

3 December 2010 | \$10

# Science



Metabolism

## SPECIAL SECTION

# Metabolism

### INTRODUCTION

- 1337 Metabolism Is Not Boring

### PERSPECTIVE

- 1338 On Getting There from Here  
*S. L. McKnight*

### REVIEWS

- 1340 The Control of the Metabolic Switch in Cancers by Oncogenes and Tumor Suppressor Genes  
*A. J. Levine and A. M. Puzio-Kuter*

- 1344 Autophagy and Metabolism  
*J. D. Rabinowitz and E. White*
- 1349 Circadian Integration of Metabolism and Energetics  
*J. Bass and J. S. Takahashi*
- 1355 Manufacturing Molecules Through Metabolic Engineering  
*J. D. Keasling*

>> *Science Translational Medicine* p. 1281 and *Science Signaling* at [www.sciencemag.org/special/metabolism/](http://www.sciencemag.org/special/metabolism/)



page 1306

### EDITORIAL

- 1287 Policy-Making Needs Science  
*Bruce Alberts*

### NEWS OF THE WEEK

- 1298 A Powerful and Perplexing New HIV Prevention Tool
- 1301 New HIV Infections Drop, But Treatment Demands Rise
- 1302 What Poison? Bacterium Uses Arsenic to Build DNA and Other Molecules  
>> *Science Express Research Article* by *F. Wolfe-Simon et al.*
- 1303 From *Science's* Online Daily News Site
- 1304 Panel Explores New Funding Pact With Washington
- 1305 With Money Tight, White House Panel Offers New Path to Energy Research
- 1305 From the *Science* Policy Blog

### NEWS FOCUS

- 1306 SCIENCE IN BRAZIL  
Brazilian Science: Riding a Gusher  
Tapping a Deep, 'Pre-Salt' Bounty  
Talented But Underfunded:  
Brazil's Future Scientists  
>> *Science Podcast*

### LETTERS

- 1316 Fishing for Data in the Ross Sea  
*L. K. Blight et al.*
- Assisted Colonization:  
Move Ahead with Models  
*M. R. Stanley Price*
- Assisted Colonization:  
Facilitate Migration First  
*J. B. Ruhl*
- Assisted Colonization:  
Protect Managed Forests  
*J. F. Fernández-Manjarrés and L. Tschanz*
- 1318 CORRECTIONS AND CLARIFICATIONS

### BOOKS ET AL.

- 1320 Brain Storm  
*R. M. Jordan-Young;*  
Delusions of Gender  
*C. Fine, reviewed by D. F. Halpern*
- 1322 Photograph 51  
*A. Ziegler, reviewed by B. Juncosa*

### POLICY FORUM

- 1324 Developing Health Workforce Capacity in Africa  
*F. S. Collins et al.*

### PERSPECTIVES

- 1326 Cryptic Links in the Ocean  
*A. Teske*  
>> *Report* p. 1375
- 1327 The DNA Damage Road Map  
*N. Friedman and M. Schuldiner*  
>> *Report* p. 1385
- 1328 Dynamic Metabolons  
*B. L. Møller*
- 1330 Opening the Cellular Poison Cabinet  
*S. J. Martin*  
>> *Report* p. 1390
- 1331 Dedicated to Memory?  
*H. Eichenbaum*  
>> *Report* p. 1408
- 1332 High-Temperature Rubber Made from Carbon Nanotubes  
*Y. Gogotsi*  
>> *Report* p. 1364

### GE PRIZE ESSAY

- 1334 A New Approach to Fluorescence Microscopy  
*M. Bates*

CONTENTS continued >>



### COVER

Autophagy, the process by which cells digest their own components, takes place in vesicles called autophagosomes (white membrane structures), the size of which are determined by the amount of a protein called Atg8. Basal amounts of Atg8 (small membrane-associated spheres) generate a small vesicle (middle left), whereas larger amounts cause formation of a full-fledged autophagosome (upper right). Autophagy provides fuel for cellular metabolism, the topic of the special section beginning on page 1337.

Painting: *David S. Goodsell*; Scientific Design: *Zhou Du and Daniel J. Klionsky*

### DEPARTMENTS

- 1283 This Week in *Science*
- 1289 Editors' Choice
- 1292 *Science* Staff
- 1297 Random Samples
- 1419 New Products
- 1420 *Science* Careers



## RESEARCH ARTICLE

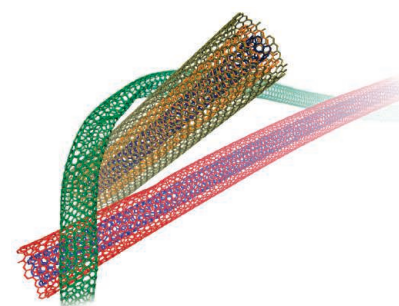
- 1359** How Learning to Read Changes the Cortical Networks for Vision and Language  
S. Dehaene et al.  
Reading changes the mind.

## REPORTS

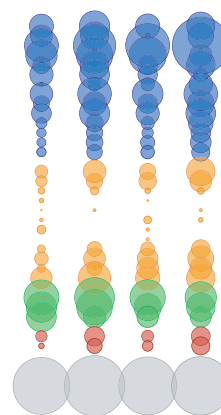
- 1364** Carbon Nanotubes with Temperature-Invariant Viscoelasticity from  $-196^{\circ}$  to  $1000^{\circ}\text{C}$   
M. Xu et al.  
A dense carbon-nanotube network shows nearly constant viscoelastic properties over an exceptionally wide temperature range.  
>> *Perspective p. 1332*
- 1368** Video-Rate Molecular Imaging in Vivo with Stimulated Raman Scattering  
B. G. Saar et al.  
Raman spectra can be acquired rapidly from samples that otherwise would scatter the usable signal.  
>> *Science Podcast*
- 1371** The Role of Particle Morphology in Interfacial Energy Transfer in CdSe/CdS Heterostructure Nanocrystals  
N. J. Borys et al.  
Single-particle spectroscopy suggests that non-uniform geometries favor efficient charge separation for light harvesting.
- 1375** A Cryptic Sulfur Cycle in Oxygen-Minimum-Zone Waters off the Chilean Coast  
D. E. Canfield et al.  
Bacterial sulfur reduction and oxidation accompanies nitrogen cycling where oxygen levels at depth are low.  
>> *Perspective p. 1326*
- 1378** Dynamical Response of the Tropical Pacific Ocean to Solar Forcing During the Early Holocene  
T. M. Marchitto et al.  
Enhanced solar activity caused the tropical Pacific to cool into a La Niña-like state during the mid-Holocene.
- 1381** Plasticity of Animal Genome Architecture Unmasked by Rapid Evolution of a Pelagic Tunicate  
F. Denoeud et al.  
A metazoan genome departs from the organization that appears rigidly established in other animal phyla.
- 1385** Rewiring of Genetic Networks in Response to DNA Damage  
S. Bandyopadhyay et al.  
A network comparison of genetic interactions mapped at two conditions reveals genetic responses to DNA damage in yeast.  
>> *Perspective p. 1327*

- 1390** BID, BIM, and PUMA Are Essential for Activation of the BAX- and BAK-Dependent Cell Death Program  
D. Ren et al.  
Proapoptotic proteins act directly on mitochondrial "gatekeeper" proteins to initiate apoptotic events during mouse development.  
>> *Perspective p. 1330*
- 1393** *Arabidopsis* Type I Metacaspases Control Cell Death  
N. S. Coll et al.  
An ancient link between cell death control and innate immune receptor function has been discovered in plants.
- 1397** An Antagonistic Pair of *FT* Homologs Mediates the Control of Flowering Time in Sugar Beet  
P. A. Pin et al.  
A homolog of a *flowering time* gene has evolved a flowering repression function, affecting the seasonal cold response in beets.
- 1400** Alleviating Neuropathic Pain Hypersensitivity by Inhibiting PKM $\zeta$  in the Anterior Cingulate Cortex  
X.-Y. Li et al.  
Nerve injury increases the activity of an enzyme in the brain and contributes to chronic pain-related cortical sensitization.
- 1404** Micro-Optical Sectioning Tomography to Obtain a High-Resolution Atlas of the Mouse Brain  
A. Li et al.  
Acquisition of light microscopic data at 1-micrometer resolution for an entire mouse brain has been developed.
- 1408** Paradoxical False Memory for Objects After Brain Damage  
S. M. McTighe et al.  
Impaired recognition may be due to treating novel objects as familiar, rather than treating familiar objects as novel.  
>> *Perspective p. 1331*
- 1410** Frequent Mutation of *BAP1* in Metastasizing Uveal Melanomas  
J. W. Harbour et al.  
A gene implicated in the control of protein degradation is mutated at high frequency in a metastatic eye cancer.
- 1413** Direct Exchange of Electrons Within Aggregates of an Evolved Syntrophic Coculture of Anaerobic Bacteria  
Z. M. Summers et al.  
Direct cell-to-cell electron transfer occurs between two related species of bacteria.

CONTENTS continued &gt;&gt;



pages 1332 &amp; 1364



pages 1326 &amp; 1375



page 1381

## SCIENCEONLINE

## SCIENCEXPRESS

[www.sciencexpres.org](http://www.sciencexpres.org)

### A Bacterium That Can Grow by Using Arsenic Instead of Phosphorus

*F. Wolfe-Simon et al.*

A bacterium has been found that can swap a toxic metalloid for a key molecular building block in its nucleic acids and enzymes.

10.1126/science.1197258

>> *News story p. 1302; Science Podcast*

### Cytoplasmic Partitioning of P Granule Components Is Not Required to Specify the Germline in *C. elegans*

*C. M. Gallo et al.*

Germ granules do not need to be segregated asymmetrically during cell division to specify germ cell fate.

10.1126/science.1193697

### Vernalization-Mediated Epigenetic Silencing by a Long Intronic Noncoding RNA

*J. B. Heo and S. Sung*

Spring flowering enabled by a winter chill is regulated by interplay between protein-coding and noncoding RNA transcripts.

10.1126/science.1197349

### Large Variations in Southern Hemisphere Biomass Burning During the Last 650 Years

*Z. Wang et al.*

Large variations in the degree of biomass burning in the Southern Hemisphere occurred during the past 650 years.

10.1126/science.1197257

### SPORE SERIES WINNER

#### Science 101: Building the Foundations for Real Understanding

*A. Thanukos et al.*

10.1126/science.1186994

## SCIENCENOW

[www.sciencenow.org](http://www.sciencenow.org)

Highlights From Our Daily News Coverage

### Black Hole May Offer Clues to Extra Dimensions

Controversial idea relies on string theory and warping of spacetime.

### The Curious Case of the Backwardly Aging Mouse

Restoring chromosome caps reverses signs of aging in rodents.

### Earth Oceans Were Homegrown

New model suggests that our seas did not come from comets and asteroids.

## SCIENCE SIGNALING

[www.sciencesignaling.org](http://www.sciencesignaling.org)

The Signal Transduction Knowledge Environment

### RESEARCH ARTICLE: The Extracellular Domain of Fibroblast Growth Factor Receptor 3 Inhibits Ligand-Independent Dimerization

*L. Chen et al.*

The extracellular domain of a receptor tyrosine kinase has opposing effects on dimerization, depending on whether ligand is present or not.

### PERSPECTIVE: Compartment-Specific Control of Signaling from a DNA-Sensing Immune Receptor

*A. Engel and G. M. Barton*

The endosomal compartment in which TLR9 is activated determines which cytokines are produced.

## GLOSSARY

Find out what BAPTA, CaSR, and DOCK mean in the world of cell signaling.

### NETWATCH: Peptide Atlas

Access a database of peptides identified in mass spectrometry analyses; in Protein Databases.

### NETWATCH: BioInteractive

Take advantage of online videos and interactive lessons for teaching and learning about biology; in Educator Sites.

## SCIENCE CAREERS

[www.sciencereers.org/career\\_magazine](http://www.sciencereers.org/career_magazine)

Free Career Resources for Scientists

### Battling Cyber Threats

*S. Carpenter*

A critical shortage of highly skilled cybersecurity technicians and engineers means wide-ranging opportunities for scientists.

### Becoming 'MacGyvers'

*S. Carpenter*

For University of Tulsa Cyber Corps students, homework means picking through Dumpsters and hacking computer systems.

### Taken for Granted: Choosing Between Science and Caring?

*B. L. Benderly*

Research suggests that many able women view careers in hard science as inimical to important values.

## SCIENCE TRANSLATIONAL MEDICINE

[www.sciencetranslationalmedicine.org](http://www.sciencetranslationalmedicine.org)

Integrating Medicine and Science

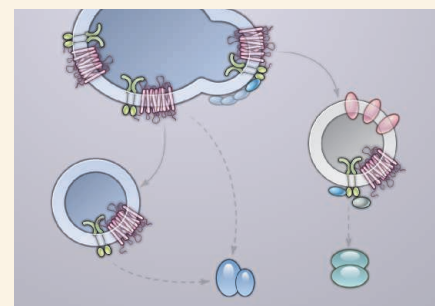
### REVIEW: The Role of JNK Proteins in Metabolism

*S. N. Vallerie and G. S. Hotamisligil*

To combat metabolic diseases, therapeutic c-Jun N-terminal kinase (JNK) inhibitors must target multiple cell types.

>> *Metabolism section p. 1337 and*

[www.sciencemag.org/special/metabolism/](http://www.sciencemag.org/special/metabolism/)



SCIENCE SIGNALING  
Trafficking inflammation.

### RESEARCH ARTICLE: IL-22+ CD4+ T Cells Are Associated with Therapeutic *Trichuris trichiura* Infection in an Ulcerative Colitis Patient

*M. J. Broadhurst et al.*

Infection with a nematode worm decreases inflammation in an ulcerative colitis patient.

### RESEARCH ARTICLE: Replacing the Enzyme $\alpha$ -L-Iduronidase at Birth Ameliorates Symptoms in the Brain and Periphery of Dogs with Mucopolysaccharidosis Type I

*A. D. Dierenfeld et al.*

Replacing  $\alpha$ -L-iduronidase at birth ameliorates symptoms in dogs with a lysosomal storage disorder.

## SCIENCE PODCAST

[www.sciencemag.org/multimedia/podcast](http://www.sciencemag.org/multimedia/podcast)

Free Weekly Show

Download the 3 December *Science* Podcast to hear about a bacterium that uses arsenic instead of phosphorus, imaging the skin in vivo, science in Brazil, and more.

## SCIENCE INSIDER

[news.sciencemag.org/scienceinsider](http://news.sciencemag.org/scienceinsider)

Science Policy News and Analysis

SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals Mail postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Copyright © 2010 by the American Association for the Advancement of Science. The title SCIENCE is a registered trademark of the AAAS. Domestic individual membership and subscription (\$1 issues): \$146 (\$74 allocated to subscription). Domestic institutional subscription (\$1 issues): \$910; Foreign postage extra: Mexico, Caribbean (surface mail) \$55; other countries (air assist delivery) \$85. First class, airmail, student, and emeritus rates on request. Canadian rates with GST available upon request, GST #1254 88122. Publications Mail Agreement Number 1069624. Printed in the U.S.A.

Change of address: Allow 4 weeks, giving old and new addresses and 8-digit account number. Postmaster: Send change of address to AAAS, P.O. Box 96178, Washington, DC 20090-6178. Single-copy sales: \$10.00 current issue, \$15.00 back issue prepaid includes surface postage; bulk rates on request. Authorization to photocopy material for internal or personal use under circumstances not falling within the fair use provisions of the Copyright Act is granted by AAAS to libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that \$20.00 per article is paid directly to CCC, 222 Rosewood Drive, Danvers, MA 01923. The identification code for Science is 0036-8075. Science is indexed in the Reader's Guide to Periodical Literature and in several specialized indexes.



ADVANCING SCIENCE, SERVING SOCIETY





## The Yin and Yang of Plant Caspases

The function of plant metacaspases, identified by limited sequence homology to the animal caspases that control cell death, has remained elusive. **Coll *et al.*** (p. 1393) have now elucidated the actions of two metacaspases in the small plant *Arabidopsis*. One metacaspase, AtMC1, promoted cell death, and the other, AtMC2, acted antagonistically to stall cell death. The results help to elucidate the mechanisms by which plants control cell survival during development and defend against pathogen attack.

## Sunny and Cool

Changes in solar output cause changes in the amount of radiation that Earth receives from the Sun, which in turn can cause climate variations. The effects of solar variations are not uniform over the globe—owing to the complexity of the climate system, larger solar fluxes may produce warming in one area but cooling in another. **Marchitto *et al.*** (p. 1378) present a record of Holocene sea surface temperature in the eastern equatorial Pacific Ocean that shows cooling as solar output increased and warming as the Sun dimmed. These temperature changes resulted from dynamical control of El Niño and La Niña episodes by solar radiative forcing of Earth's climate.

## Reading, Writing, and Face Recognition

Reading, not to mention writing and texting, is a relatively recent invention, and hence it is believed that a preliterate brain must adapt on the fly, so to speak, in learning how to process

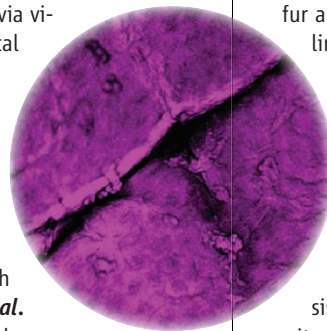
written words, rather than being able to rely upon evolutionarily ancient modifications of the visual system pathways. **Dehaene *et al.*** (p. 1359, published online 11 November) examined the neural response to a range of visual stimuli in three groups: illiterate adults, adults who learned to read as children, and adults who learned to read as adults. Reading induced a greater facility in processing horizontally oriented stimuli at early stages in the visual pathway and was also associated with the appearance of an area specialized for words. This gain of function appeared to occur at a cost—the area in the temporal cortex devoted to face processing shrank.

## Shake It to Wake It

Viscoelastic materials combine the recoverable stretchiness found in elastic materials with the slow-flowing behavior of a thick fluid, like honey. When subjected to an oscillatory motion, the response will depend on the frequency. At low frequencies, the viscous behavior will dominate and lead to a dissipation of the applied energy as heat, while at fast frequencies the elastic behavior dominates. **Xu *et al.*** (p. 1364; see the Perspective by **Gogotsi**) developed a viscoelastic material with an exceptionally broad operating temperature range, based on a network of carbon nanotubes. The responsiveness of the material was probably caused by the “zipping” and “un-zipping” of the nanotubes at points of contact.

## Skin-Deep Raman Spectroscopy

Raman spectroscopy allows for molecular identification via vibrational spectra at optical wavelengths. However, if the optical signal is scattered, as occurs when trying to image tissue, the signal becomes very weak, and it becomes difficult to image a sample with high time resolution. **Saar *et al.*** (p. 1368) now show that by improving the optics and electronics of the acquisition of the backscattered signal, stimulated Raman scattering spectroscopy can be performed at video rates on human skin, which should enable label-free studies of tissues and, for example, the tracking of the delivery of a drug.



## An Upside of Asymmetry

Advances in synthetic techniques have enabled the preparation of nanometer-scale semiconductors in a wide range of precise shapes and sizes, including core-shell morphologies that layer several different materials in the same particle. Such two-in-one motifs are promising for light-harvesting applications because they allow optically induced charge separation across the internal interface. **Borys *et al.*** (p. 1371) studied a series of rod-shaped cadmium sulfide–cadmium selenide hybrid particles using single-particle–resolved optical spectroscopy and found that smooth versus bulbous geometries produced distinct emission spectra. Further analysis of more complex, tetrapodal particles (with four arms aligned tetrahedrally) suggested that nonuniform geometries facilitate interfacial charge transfer by reducing the likelihood of electronic band misalignment.

## Cryptic Sulfur Cycling

Aerobic bacteria and ocean circulation patterns control the formation and distribution of oxygen-minimum zones at moderate depth in the oceans. These habitats host microorganisms that thrive on other metabolic substrates in the absence of oxygen—most commonly, metabolizing thermodynamically favorable nitrogen compounds like nitrate. Off the coast of Chile, however, **Canfield *et al.*** (p. 1375, published online 11 November; see the Perspective by **Teske**) suggest that bacteria may often reduce sulfate as well. Metagenomic sequencing revealed the presence of both sulfate-reducing and sulfide-oxidizing bacteria. With the coincidence of sulfate and nitrate reduction, the sulfur and nitrogen cycles may be intimately linked; for example, sulfate reduction could provide nitrogen-rich ammonium for bacteria that ultimately transform it into nitrogen gas.

## Deadly Trio

The proteins BAX and BAK act as a key decision point, regulating apoptosis by controlling the permeability of the mitochondrial outer membrane. Evidence has been presented for two mechanisms of activation of BAX and BAK: an indirect mechanism where proapoptotic proteins neutralize the antiapoptotic effects of the protein BCL-2 and its relatives; or direct activation of BAX and BAK by BIM, BID, or PUMA. Analysis of the

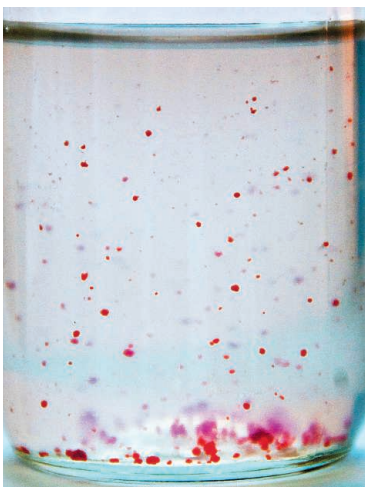
*Continued on page 1285*

Continued from page 1283

situation in vivo is complicated by the overlapping function of BIM, BID, and PUMA. **Ren et al.** (p. 1390; see the Perspective by **Martin**) thus analyzed triple-knockout mice lacking BIM, BID, and PUMA. Apoptosis during mouse development required a direct effect of one of these proteins to activate BAX or BAK, thereby promoting cell death.

## Better Brain Maps

A high-resolution atlas of the complete neuronal connectivity in a whole brain should fundamentally advance our understanding of the organization and function of animal nervous systems. Now, **A. Li. et al.** (p. 1404, published online 4 November) describe an automated system, micro-optical sectioning tomography, that allowed three-dimensional mapping of the morphology and spatial location of neurons and traces of neurites in a whole, intact mouse brain.



## Wired for Life

Syntrophic bacteria live on the metabolic by-products of a partner species. The exchange of the by-products accompanies a flow of electrons in the opposite direction that helps some species grow in conditions that would otherwise be unfavorable. In mixed anaerobic cultures of two related *Geobacter* species, **Summers et al.** (p. 1413) observed that one species evolved to promote the transfer of electrons directly to the other, in large aggregated cell clusters, without coupling to common anaerobic by-products such as hydrogen or formate. Selection pressures in nine parallel populations all resulted in a point mutation that truncated a protein involved in the production of small hairlike projections involved in intercellular communication—pili—and indirectly increased the expression of a c-type multiheme cytochrome responsible for extracellular electron transfer. The evolved aggregates

were conductive, suggesting that the direct exchange of electrons between partner species is a possible alternative route to anaerobic syntrophy rather than interspecies hydrogen transfer; indeed, deleting a gene that encodes a hydrogenase involved in hydrogen transfer conferred a growth advantage in the cocultures.

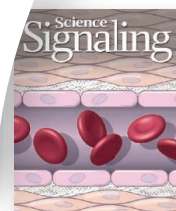
## Novel or Familiar?

Amnesia is characterized by a number of memory deficits, including the apparent inability to distinguish between novel and familiar stimuli. **McTighe et al.** (p. 1408; see the Perspective by **Eichenbaum**) observed that the recognition memory of brain-damaged rats in a standard model of amnesia was impaired not because previously experienced objects seemed to be novel, but because objects not previously experienced seemed to be familiar. Furthermore, simply placing the animal in a visually deprived environment during the delay, reducing visual interference, completely rescued the impairment. This counterintuitive finding contradicts the predominant “multiple memory systems” model in which amnesia is usually considered and forces a reconsideration of fundamental assumptions underlying our understanding of amnesia.

## An Eye on Metastasis

Despite the considerable progress being made in elucidating the cell biology of metastasis, little is known about the genetic alterations that promote metastasis of human tumors, the cause of most cancer deaths. A potentially important clue now emerges from the work of **Harbour et al.** (p. 1410, published online 4 November), who used an exome-sequencing approach to search for genetic mutations in uveal melanomas, an eye cancer associated with a high rate of fatal metastasis. Remarkably, over 80% of tumor samples with a high metastatic risk had inactivating somatic mutations in the gene encoding BAP1 (BRCA1-associated protein 1), a nuclear protein involved in controlling protein degradation. Thus, in this tumor type, mutational inactivation of BAP1 may be a key event in the acquisition of metastatic competence.

Call for  
Papers



## Science Signaling

**Science Signaling**, from the publisher of **Science**, AAAS, features top-notch, peer-reviewed, original research weekly. Submit your manuscripts in the following areas of cellular regulation:

- Biochemistry
- Bioinformatics
- Cell Biology
- Development
- Immunology
- Microbiology
- Molecular Biology
- Neuroscience
- Pharmacology
- Physiology and Medicine
- Systems Biology

Subscribing to **Science Signaling** ensures that you and your lab have the latest cell signaling resources. For more information visit [www.ScienceSignaling.org](http://www.ScienceSignaling.org)

### Chief Scientific Editor

**Michael B. Yaffe, M.D., Ph.D.**

Associate Professor, Department of Biology  
Massachusetts Institute of Technology

### Editor

**Nancy R. Gough, Ph.D.**  
AAAS

Submit your research at:  
[www.sciencesignaling.org/  
about/help/research.dtl](http://www.sciencesignaling.org/about/help/research.dtl)

Science Signaling





Bruce Alberts is Editor-in-Chief of *Science*.

## Policy-Making Needs Science

OVER THE LONG RUN, ANY NATION THAT MAKES CRUCIAL DECISIONS WHILE IGNORING SCIENCE is doomed. Consider, for example, the decision about how much arsenic should be allowed in drinking water supplies. There is no one “right answer” to this or many other policy questions, but it is critical that national legislation be based on what science knows about potential harm. It is therefore disturbing that so many lawmakers elected to the new U.S. Congress reject the overwhelming scientific consensus with respect to human-induced climate change. It will be difficult to make wise choices with such attitudes. The question now facing the United States is not only how to effectively reinject the facts of climate science back into the core of this particular debate, but also how to ensure that good science underlies all legislative decisions.

For 12 years, I served as the president of the U.S. National Academy of Sciences.

As part of a larger nongovernmental organization known as the National Academies, it produces more than 200 reports a year, aimed at making the current scientific consensus on important issues available to policy-makers and the public. In major reports released this spring, the National Academies strongly reiterated its position that climate change, caused largely by human activities, poses significant risks to the world’s future.\* This conclusion is nevertheless challenged by numerous politicians, as well as by a substantial fraction of the public. There is only one effective solution for this type of problem: Scientists must make both science education and community outreach a much more central part of the scientific culture.

Most Americans have never met a scientist, and despite having been “taught science” at school, most have no real idea of how a scientific consensus is reached through continuous open debate and experiment. Every adult should have a base of scientific understanding about how the world works. But understanding the process through which scientific knowledge develops is equally critical. By the end of any introductory college science class—which can be an adult’s final exposure to science—a student should have a realistic understanding of the nature of science. Scientists are taught to challenge authority, and their responsible challenges to a consensus help science advance. Thus, adults should expect to find some scientists who disagree with the scientific consensus on an issue. And they should appreciate why a strong scientific consensus, such as that about climate change, must nevertheless form the basis for making wise personal and community decisions, representing by far the best bet for predicting the future consequences of present actions.

In addition to education, an energetic community outreach to schools, the public, and decision-makers is key. Both established scientists and those in training can be highly effective in putting a human face on science and conveying optimistic, honest attitudes toward grappling with society’s problems. Week-long science festivals, to which local institutions based on science and engineering contribute ideas and personnel, should become an annual event at hundreds of sites around the nation. And programs that encourage and facilitate outreach into nonscientific communities need to become a standard part of every university and science-based industrial establishment.

The environment in which decisions are made in a democracy will always be highly politicized, but it is crucial that both sides of any argument pay close attention both to what science knows and how that knowledge has been gained. Attaining this goal in every nation will require that scientists vigorously reach out to their communities, informing them not only about their new discoveries, but also about the path they took to get there.†

— Bruce Alberts

10.1126/science.1200613

\*<http://americasclimatechoices.org>. †T. E. Bowman *et al.*, *Science* **330**, 1044 (2010).







ECOLOGY

## Migration-Based Monitoring

Counts of birds passing traditional hawk-watching sites provide a means for estimating population trends in migrant raptors. To gauge the effectiveness of such efforts, Farmer *et al.* explore the degree to which the existing network of sites could sample the autumn tracks of 57 adult ospreys (*Pandion haliaetus*). Birds from four breeding areas across the United States were fitted with satellite transmitters. An average of 1.5 relocations per day were observed per bird. To analyze flight trajectories, the authors identified migration paths that connected locations with 3-km- or 6-km-wide corridors. Across North America, 12% of the narrow and 23% of the broader pathways intersected one or more active watch sites; these values rose to 21 and 36% in the eastern region (through which the majority of the continent's population probably passes). Models that assume a conditional random walk between consecutive positional fixes estimate that 95% of the birds' utilization distributions cross at least one observation area. Disparities in mean regional detection probabilities for individuals (east, 34%; midwest, 5.8%; northwest, 4.7%) reflect the relative distribution of hawk watches. The satellite tracks also show southbound ospreys concentrating along narrow fronts and avoiding large water crossings. These results suggest that migration counts do offer a practical means of monitoring populations of the North American osprey and perhaps many other hawks. — SJS

*Auk* 127, 863 (2010).

EVOLUTION

## Good for You, Good for Me, Too

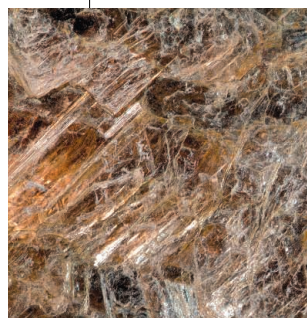
In most examples of cooperative breeding, reproductive individuals are assisted by closely related helpers. Although helpers sacrifice their own ability to reproduce, they obtain an indirect fitness benefit from improving survival in their kin (kin theory). Increasingly, however, cooperative breeding has been revealed among unrelated individuals. In greater anis (*Crotophaga major*), multiple pairs of birds will cooperate to build nests and raise and defend young. Riehl has now shown that these pairs are relatively unrelated, thus cooperation cannot be explained by kin theory. Instead, she finds that the individual fitness of all the pairs is increased when pairs cooperate, despite initial reproductive competition generated during egg-laying. Three pair groups were able to obtain and defend prime nesting sites better than two pair groups, and pairs attempting to breed alone failed in all observed instances. Furthermore, there was no cost to individual fitness on the basis of the number of eggs laid, most likely because females changed their laying order across years. In unrelated cooperative breeders, what's good for "the many" is most likely also good for "the one." — SNV

*Proc. R. Soc. London Ser. B* 10.1098/rspb.2010.1752 (2010).

GEOCHEMISTRY

## Reflecting on Mica

Trace metals in soils and sediment predominantly exist as either tiny mineral grains or mobile aqueous ions in pore water. In both cases, adsorption and desorption reactions at



the surface of mineral grains control their fate and transport through the subsurface. Because the size of ions and strength of the surrounding hydration shell are highly variable, predicting the way in which metals are distributed even at

one specific mineral/water interface is challenging. To systematically address this question, Lee *et al.* measured—using an X-ray reflectivity technique sensitive enough to determine the thickness of a single layer of water—the

*Continued on page 1291*

BIOCHEMISTRY

## Acting Like Actin

In a dividing cell, DNA must be replicated and accurately partitioned between the two daughter cells. In eukaryotes, microtubules, which are linked to the centromeric DNA by kinetochores, separate the replicated chromosomes with the help of motor proteins. Prokaryotic plasmid-partitioning systems are similar in that they involve an adaptor protein that links DNA to a filament-forming protein; however, no motor proteins are involved, and nucleotide-hydrolysis dependent filament dynamics are responsible for movement. To gain insight into the segregation of the *Bacillus thuringiensis* pBTaxis plasmid, Aylett *et al.* determined the

structure of two protofilaments of the tubulin/FtsZ-like protein, TubZ, which mediates plasmid segregation. A combination of crystallography and electron microscopy revealed that instead of forming tubulin-like straight protofilaments that organize into microtubules, TubZ forms twisted double filaments that can bundle, reminiscent of structures formed by ParM, an actin-like prokaryotic partitioning filament. That two disparate proteins have evolved to perform a similar function using similar superstructures suggests strong evolutionary constraints on partitioning systems. — VV

*Proc. Natl. Acad. Sci. U.S.A.* 107, 19766 (2010).

Continued from page 1289

manner in which divalent metal ions adsorbed on a hydrated muscovite surface. As expected, metal ions with weak hydration shells lost water and bound tightly to muscovite within just 2.5 Å; stronger hydration shells remained intact, pushing other metal ions further out (3.5 to 4.5 Å from the surface). The reflectivity profiles also revealed, surprisingly, an unexpected additional broad layer of metal ions 5 to 10 Å away from the mineral surface. Computational models of similar interfaces may need to be adjusted to account for this extended layer. — NW

*Langmuir* **26**, 16647 (2010).

## PHYSICS

## Hiding in the Pseudogap

When cuprate superconductors, which can conduct electricity without resistance up to temperatures on the order of 150 K, were first discovered almost 25 years ago, it was expected that the explanation for their extraordinary properties was around the corner and would lead to even higher transition temperatures  $T_c$ . Today this mechanism remains a mystery; however, most researchers agree that the so-called pseudogap region, which lies above  $T_c$  but is characterized by a non-zero excitation gap that disappears at a higher temperature  $T^*$ , is key to understanding the entire phase diagram. Along the doping axis, the pseudogap region borders an antiferromagnetic phase, the remnants of which can be detected by neutron scattering as a resonance centered around the corner of the Brillouin zone. Li *et al.* use polarized neutron scattering to detect another magnetic excitation that is present at all wave vectors and, surprisingly, has an integrated spectral weight an order of magnitude higher than that of the resonance. Because the studied compound,  $\text{HgBa}_2\text{CuO}_{4+\delta'}$  is thought to be representative of the cuprates, and the new magnetic excitation appears at the same  $T^*$  determined from prior transport and neutron diffraction measurements, it is tempting to interpret this result as a corroborating piece of evidence that a true phase transition, rather than a crossover, takes place at  $T^*$ . — JS

*Nature* **468**, 283 (2010).



is absorbed by the sea and by the terrestrial biosphere. Studies have suggested that North America constituted a terrestrial carbon sink during the past several decades, although how much carbon it has accumulated remains debatable. Crevoisier *et al.* have now estimated land-atmosphere fluxes across North America during the period from 2004 to 2006 by balancing the inflow and outflow of carbon dioxide from the troposphere, using a simple budgeting approach and vertical profiles of atmospheric carbon dioxide to construct the distribution over the continent in three dimensions. They find that the coterminous United States provided a moderate sink and that the distribution of carbon uptake agrees well with agricultural and climate patterns during that time. Their technique thus also provides a potential independent method to link regional carbon uptake to climate drivers. — HJS

*Proc. Natl. Acad. Sci. U.S.A.* **107**, 18348 (2010).

## SIGNAL TRANSDUCTION

## Cell as Corporation

Systems biology provides insight into the complex regulatory networks within a cell, with the hope that such analysis will yield insight into how biological control systems work and how they may be modified for therapeutic benefit. Some network models suggest that the extent of connectivity of a transcriptional regulator indicates the essentiality of that protein in that system. Bhardwaj *et al.* propose an alternative view. They organized transcription factors in yeast or bacteria in a pyramidal hierarchy and then used information regarding the effects of the various factors on cell fitness to determine that the importance of a transcription factor is more related to its placement in the hierarchy than to its total direct connectivity to target genes. Thus, proteins at the top of the hierarchy exerted the greatest influence

over cell fitness. The relative ease of testing network perturbations in biological systems suggests that this type of analysis may also be useful in understanding similar corporate, government, or social networks, where one cannot so easily intervene. — LBR

*Sci. Signal.* **3**, ra79 (2010).

## CLIMATE SCIENCE

## The American Sink

Understanding how fast the concentration of carbon dioxide in the atmosphere will rise as we continue to burn fossil fuels depends on how well we know what fraction of emissions

## Call for Papers

## Science Translational Medicine

## Integrating Medicine and Science

The new journal from the publisher of *Science* stands at the forefront of the unprecedented and vital collaboration between basic scientists and clinical researchers.

- Cardiovascular Disease
- Neuroscience/Neurology/Psychiatry
- Infectious Diseases
- Cancer
- Health Policy
- Bioengineering
- Chemical Genomics/Drug Discovery
- Other Interdisciplinary Approaches to Medicine

Submit your research at  
[www.submit2scitranslmed.org](http://www.submit2scitranslmed.org)



Chief Scientific Adviser  
**Elias A. Zerhouni, M.D.**  
Former Director,  
National Institutes of Health



[ScienceTranslationalMedicine.org](http://ScienceTranslationalMedicine.org)



MANAGING EDITOR, RESEARCH JOURNALS **Katrina L. Kelner**  
DEPUTY EDITORS **R. Brooks Hanson, Barbara R. Jasny, Andrew M. Sugden**

EDITORIAL SENIOR EDITORS/COMMENTARY **Lisa D. Chong, Brad Wible**; SENIOR EDITORS **Gilbert J. Chin, Pamela J. Hines, Paula A. Kiberstis (Boston), Marc S. Lavine (Toronto), Beverly A. Purnell, L. Bryan Ray, Guy Riddiough, H. Jesse Smith, Phillip D. Szurimi (Tennessee), Valda Vinson, Jake S. Yeston**; ASSOCIATE EDITORS **Kristen L. Mueller, Jelena Stajic, Sacha Vignieri, Nicholas S. Wigginton, Laura M. Zahn (San Diego)**; BOOK REVIEW EDITOR **Sherman J. Suter**; ASSOCIATE LETTERS EDITOR **Jennifer Silly**; EDITORIAL MANAGER **Cara Tate**; SENIOR COPY EDITORS **Jeffrey E. Cook, Cynthia Howe, Harry Jach, Lauren Kmeic, Barbara P. Ordway, Trista Wagoner**; COPY EDITOR **Chris Filiatreau**; EDITORIAL COORDINATORS **Carolyn Kyle, Beverly Shields**; PUBLICATIONS ASSISTANTS **Ramatoulaye Diop, Joi S. Granger, Emily Guise, Jeffrey Hearn, Michael Hicks, Lisa Johnson, Scott Miller, Jerry Richardson, Jennifer A. Seibert, Brian White, Anita Wynn**; EDITORIAL ASSISTANTS **Emily C. Horton, Patricia M. Moore, Marlene Weinberg**; EXECUTIVE ASSISTANT **Alison Crawford**; ADMINISTRATIVE SUPPORT **Maryrose Madrid**; EDITORIAL FELLOW **Melissa R. McCartney**

EDITORIAL DIRECTOR, WEB AND NEW MEDIA **Stewart Wills**; SENIOR WEB EDITOR **Tara S. Marathe**; WEB EDITOR **Robert Frederick**; RESEARCH ASSOCIATE **Corinna Choo**; WEB DEVELOPMENT MANAGER **Martyn Green**; WEB DEVELOPER **Andrew Whitesell**; INTERN **Sophia Cai**

NEWS DEPUTY NEWS EDITORS **Robert Coontz, David Grimm (Online), Eliot Marshall, Jeffrey Mervis, Leslie Roberts**; CONTRIBUTING EDITORS **Elizabeth Colutta, Polly Shulman**; NEWS WRITERS **Yudhiti Bhattacharjee, Adrian Cho, Jennifer Cohn, Jocelyn Kaiser, Richard A. Kerr, Eli Kintisch, Greg Miller, Elizabeth Pennisi, Lauren Schenckman, Robert F. Service (Pacific NW), Erik Stokstad**; WEB DEVELOPER **Daniel Berger**; INTERN **Kristen Minogue**; CONTRIBUTING CORRESPONDENTS **Jon Cohen (San Diego, CA), Daniel Ferber, Ann Gibbons, Sam Kean, Andrew Lawler, Mitch Leslie, Charles C. Mann, Virginia Morell, Gary Taubes**; COPY EDITORS **Linda B. Felaco, Melvin Gatling, Melissa Raimondi**; ADMINISTRATIVE SUPPORT **Scheraine Mack**; BUREAUS **San Diego, CA: 760-942-3252, FAX 760-942-4979; Pacific Northwest: 503-963-1940**

PRODUCTION DIRECTOR **Wendy K. Shank**; ASSISTANT MANAGER **Rebecca Doshi**; SENIOR SPECIALISTS **Steve Forrester, Chris Redwood, Anthony Rosen**; PREFLIGHT DIRECTOR **David M. Tompkins**; MANAGER **Marcus Spiegler**; SPECIALIST **Jason Hillman**

ART DIRECTOR **Yael Fitzpatrick**; ASSOCIATE ART DIRECTOR **Laura Creveling**; SENIOR ILLUSTRATORS **Chris Bickel, Katharine Suttiff**; ILLUSTRATOR **Yana Hammond**; SENIOR ART ASSOCIATES **Holly Bishop, Preston Huey, Nayomi Kevitiyagala**; ART ASSOCIATES **Kay Engman, Matthew Twombly**; PHOTO EDITOR **Leslie Blizard**

## SCIENCE INTERNATIONAL

EUROPE (science@science-int.co.uk) EDITORIAL: INTERNATIONAL MANAGING EDITOR **Andrew M. Sugden**; SENIOR EDITOR/COMMENTARY **Julia Fahrenkamp-Uppenbrink**; SENIOR EDITORS **Caroline Ash, Stella M. Hurtle, Ian S. Osborne, Peter Stern**; ASSOCIATE EDITOR **Maria Cruz**; LOCUM EDITOR **Helen Pickersgill**; EDITORIAL SUPPORT **Samantha Hogg, Alice Whaley**; ADMINISTRATIVE SUPPORT **John Cannell, Janet Clements, Louise Hartwell**; NEWS: EUROPE NEWS EDITOR **John Travis**; DEPUTY NEWS EDITOR **Daniel Clerly**; CONTRIBUTING CORRESPONDENTS **Michael Balter (Paris), John Bohnannon (Vienna), Martin Enserink (Amsterdam and Paris), Gretchen Vogel (Berlin)**; INTERN **Jennifer Carpenter**

LATIN AMERICA CONTRIBUTING CORRESPONDENT **Antonio Regalado**

ASIA Japan office: Asca Corporation, Tomoko Furusawa, Rustic Bldg. 7F, 77 Tenjin-cho, Shinjuku-ku, Tokyo 162-0808, Japan; +81 3 6802 4616, FAX +81 3 6802 4615, inquiry@sciencemag.jp; ASIA NEWS EDITOR **Richard Stone (Beijing: rstone@aaas.org)**; CONTRIBUTING CORRESPONDENTS **Dennis Normile [Japan: +81 (0) 3 3391 0630, FAX +81 (0) 3 5936 3531; dnornile@gol.com]**; **Hao Xin [China: cindyhao@gmail.com]**; **Pallava Bagla [South Asia: +91 (0) 11 2271 2896; pbagla@vsnl.com]**

FULFILLMENT SYSTEMS AND OPERATIONS (membership@aaas.org); DIRECTOR **Waylon Butler**; CUSTOMER SERVICE SUPERVISOR **Pat Butler**; SPECIALISTS **Latoya Casteel, LaVonda Crawford, Vicki Linton, April Marshall**; DATA ENTRY SUPERVISOR **Cynthia Johnson**; SPECIALISTS **Shirlene Hall, Tarrika Hill, William Jones**

BUSINESS OPERATIONS AND ADMINISTRATION DIRECTOR **Deborah Rivera-Wienhold**; BUSINESS SYSTEMS AND FINANCIAL ANALYSIS DIRECTOR **Randy Yi**; MANAGER, BUSINESS ANALYSIS **Eric Knott**; MANAGER, BUSINESS OPERATIONS **Jessica Tierney**; FINANCIAL ANALYSTS **Priti Pamnani, Celeste Troxler**; RIGHTS AND PERMISSIONS: ADMINISTRATOR **Emilie David**; ASSOCIATE **Elizabeth Bandler**; MARKETING DIRECTOR **Jan King**; MARKETING MANAGERS **Allison Pritchard, Alison Chandler, Julianne Wielga**; MARKETING ASSOCIATES **Aimee Aponte, Mary Ellen Crowley, Wendy Wise**; SENIOR MARKETING EXECUTIVE **Jennifer Reeves**; DIRECTOR, SITE LICENSING **Tom Ryan**; DIRECTOR, CORPORATE RELATIONS **Eileen Bernadette Moran**; PUBLISHER RELATIONS, eRESOURCES SPECIALIST **Kiki Forsythe**; SENIOR PUBLISHER RELATIONS SPECIALIST **Catherine Holland**; PUBLISHER RELATIONS, EAST COAST **Phillip Smith**; PUBLISHER RELATIONS, WEST COAST **Philip Tsolakis**; FULFILLMENT SUPERVISOR **Iqoo Edim**; FULFILLMENT COORDINATOR **Carrie MacDonald, Destiny Pinson**; MARKETING MANAGER **Christina Schlecht**; MARKETING ASSOCIATE **Laura Tutino**; ELECTRONIC MEDIA: MANAGER **Lizabeth Harman**; PROJECT MANAGER **Trista Snyder**; ASSISTANT MANAGER **Lisa Stanford**; SENIOR PRODUCTION SPECIALISTS **Ryan Atkins, Christopher Coleman**; COMPUTER SPECIALIST **Walter Jones, Kai Zhang**; PRODUCTION SPECIALISTS **Antoinette Hodal, Nichole Johnston, Kimberly Oster**; DIRECTOR, WEB AND NEW MEDIA **Will Collins**

ADVERTISING DIRECTOR, WORLDWIDE AD SALES **Bill Moran**

COMMERCIAL EDITOR **Sean Sanders**; 202-326-6430

ASSISTANT COMMERCIAL EDITOR **Tianna Hicklin** 202-326-6463

PROJECT DIRECTOR, OUTREACH **Brianna Blaser**

PRODUCT (science\_advertising@aaas.org); MIDWEST **Rick Bongiovanni**: 330-405-7080, FAX 330-405-7081; EAST COAST/E. CANADA **Laura Faraday**: 508-747-9395, FAX 617-507-8189; WEST COAST/W. CANADA **Lynne Stickrod**: 415-931-9782, FAX 415-520-6940; UK/EUROPE/ASIA **Roger Goncalves**: TEL/FAX +41 43 243 1358; JAPAN **ASCA Corporation**, Nanako Ide +81 (0) 3 6802 4616, FAX +81 (0) 3 6802 4615; ads@sciencemag.jp; SENIOR TRAFFIC ASSOCIATE **Deandra Simms**

WORLDWIDE ASSOCIATE DIRECTOR OF SCIENCE CAREERS **Tracy Holmes**: +44 (0) 1223 326525, FAX +44 (0) 1223 326532

CLASSIFIED (advertise@sciencecareers.org); U.S.: MIDWEST/WEST COAST/SOUTH CENTRAL/CANADA **Tina Burks**: 202-326-6577; EAST COAST/INDUSTRY **Elizabeth Early**: 202-326-6578; SALES ADMINISTRATOR: **Marci Gallun**; SALES COORDINATORS **Rohan Edmonson, Shirley Young**; EUROPE/ROW SALES: **Susanne Kharraz, Dan Pennington, Alex Palmer**; SALES ASSISTANT **Lisa Patterson**; JAPAN **ASCA Corporation**, Jie Chin +81 (0) 3 6802 4616, FAX +81 (0) 3 6802 4615; careerads@sciencemag.jp; ADVERTISING SUPPORT MANAGER **Karen Foote**: 202-326-6740; ADVERTISING PRODUCTION OPERATIONS MANAGER **Deborah Tompkins**; SENIOR PRODUCTION SPECIALIST/GRAPHIC DESIGNER **Amy Hardcastle**; PRODUCTION SPECIALIST **Yuse Lajiminmuhip**; SENIOR TRAFFIC ASSOCIATE **Christine Hall**

AAAS BOARD OF DIRECTORS RETIRING PRESIDENT, CHAIR **Peter C. Agre**; PRESIDENT **Alisa Huang**; PRESIDENT-ELECT **Nina Fedoroff**; TREASURER **David E. Shaw**; CHIEF EXECUTIVE OFFICER **Alan I. Leshner**; BOARD **Linda P. B. Katehi, Nancy Knowlton, Stephen Mayo, Cherry A. Murray, Julia M. Phillips, Sue V. Rosser, David D. Sabatini, Thomas A. Woolsey**

SUBSCRIPTION SERVICES For change of address, missing issues, new orders and renewals, and payment questions: 866-434-AAAS (2227) or 202-326-6417, FAX 202-842-1065. Mailing addresses: AAAS, P.O. Box 96178, Washington, DC 20090-6178 or AAAS Member Services, 1200 New York Avenue, NW, Washington, DC 20005

INSTITUTIONAL SITE LICENSES please call 202-326-6755 for any questions or information

REPRINTS: Author Inquiries 800-635-7181

Commercial Inquiries 803-359-4578

PERMISSIONS 202-326-7074, FAX 202-682-0816

MEMBER BENEFITS AAAS/Barnes&Noble.com bookstore www.aaas.org/bn; AAAS Online Store www.apisource.com/aaas/ code MKB6; AAAS Travels: Betchart Expeditions 800-252-4910; Apple Store www.apple.com/epstore/aaas; Bank of America MasterCard 1-800-833-6262 priority code FAA3YU; Cold Spring Harbor Laboratory Press Publications www.cshlpress.com/affiliates/aaas.htm; GEICO Auto Insurance www.geico.com/landingpage/gos1.htm?logo=17624; Hertz 800-654-2200 CDP#343457; Office Depot https://bsd.officedepot.com/portallogin.do; Seabury & Smith Life Insurance 800-424-9883; Subaru VIP Program 202-326-6417; VIP Moving Services www.vipmayflower.com/domestic/index.html; Other Benefits: AAAS Member Services 202-326-6417 or www.aaasmember.org.

science\_editors@aaas.org (for general editorial queries)  
science\_letters@aaas.org (for queries about letters)  
science\_reviews@aaas.org (for returning manuscript reviews)  
science\_bookrevs@aaas.org (for book review queries)

Published by the American Association for the Advancement of Science (AAAS), Science serves its readers as a forum for the presentation and discussion of important issues related to the advancement of science, including the presentation of minority or conflicting points of view, rather than by publishing only material on which a consensus has been reached. Accordingly, all articles published in Science—including editorials, news and comment, and book reviews—are signed and reflect the individual views of the authors and not official positions of view adopted by AAAS or the institutions with which the authors are affiliated.

AAAS was founded in 1848 and incorporated in 1874. Its mission is to advance science, engineering, and innovation throughout the world for the benefit of all people. The goals of the association are to: enhance communication among scientists, engineers, and the public; promote and defend the integrity of science and its use; strengthen support for the science and technology enterprise; provide a voice for science on societal issues; promote the responsible use of science in public policy; strengthen and diversify the science and technology workforce; foster education in science and technology for everyone; increase public engagement with science and technology; and advance international cooperation in science.

## INFORMATION FOR AUTHORS

See pages 352 and 353 of the 15 January 2010 issue or access www.sciencemag.org/about/authors

## SENIOR EDITORIAL BOARD

**John I. Brauman**, Chair, Stanford Univ.  
**Richard Losick**, Harvard Univ.  
**Linda Partridge**, Univ. College London  
**Michael S. Turner**, University of Chicago

## BOARD OF REVIEWING EDITORS

**Adriano Aguzzi**, Univ. Hospital Zürich  
**Takuzo Aida**, Univ. of Tokyo  
**Sonia Altizer**, Univ. of Georgia  
**David Altshuler**, Broad Institute  
**Arturo Alvarez-Buylla**, Univ. of California, San Francisco  
**Richard Amasino**, Univ. of Wisconsin, Madison  
**Angelika Amon**, MIT  
**Kathryn Anderson**, Memorial Sloan-Kettering Cancer Center  
**Siv G. E. Andersson**, Uppsala Univ.  
**Peter Andolfatto**, Princeton Univ.  
**Meinrat O. Andreae**, Max Planck Inst., Mainz  
**John A. Bargh**, Yale Univ.  
**Ben Barres**, Stanford Medical School  
**Marisa Bartolomeo**, Univ. of Penn. School of Med.  
**Jordi Bascompte**, Estación Biológica de Doñana, CSIC  
**Facundo Batista**, London Research Inst.  
**Ray H. Baughman**, Univ. of Texas, Dallas  
**David Baum**, Univ. of Wisconsin  
**Yasmine Belkaid**, NIAID, NIH  
**Stephen J. Benkovic**, Penn State Univ.  
**Gregory C. Berzox**, Stanford Univ.  
**Tom Bisessing**, Wageningen Univ.  
**Mina Bissell**, Lawrence Berkeley National Lab  
**Peer Bork**, EMBL  
**Robert W. Boyd**, Univ. of Rochester  
**Paul M. Brakefield**, Leiden Univ.  
**Christian Büchel**, Universitätsklinikum Hamburg-Eppendorf  
**Joseph A. Burns**, Cornell Univ.  
**William P. Buttz**, Population Reference Bureau  
**Mats Carlsson**, Univ. of Oslo  
**Mildred Cho**, Stanford Univ.  
**David Clapham**, Children's Hospital, Boston  
**David Clary**, Oxford University  
**J. M. Claverie**, CNRS, Marseille  
**Jonathan D. Cohen**, Princeton Univ.  
**Andrew Cossins**, Univ. of Liverpool  
**Alan Cowman**, Walter & Eliza Hall Inst.  
**Robert H. Crabtree**, Yale Univ.  
**Wolfgang Cramer**, Potsdam Inst. for Climate Impact Research

**F. Fleming Crim**, Univ. of Wisconsin  
**Jeff L. Dangl**, Univ. of North Carolina  
**Tom Daniel**, Univ. of Washington  
**Stamatis Dehaene**, Collège de France  
**Emmanouil T. Dermitzakis**, Univ. of Geneva Medical School  
**Robert Desimone**, MIT  
**Claude Desplan**, New York Univ.  
**Ap Dijksterhuis**, Radboud Univ. of Nijmegen  
**Dennis Discher**, Univ. of Pennsylvania  
**Scott C. Doney**, Woods Hole Oceanographic Inst.  
**Jennifer A. Doudna**, Univ. of California, Berkeley  
**Julian Downward**, Cancer Research UK  
**Bruce Dunn**, Univ. of California, Los Angeles  
**Christopher Dye**, WHO  
**Michael B. Elowitz**, Calif. Inst. of Technology  
**Gerhard Ertl**, Fritz-Haber-Institut, Berlin  
**Barry Everitt**, Univ. of Cambridge  
**Paul G. Falkowski**, Rutgers Univ.  
**Ernst Fehr**, Univ. of Zurich  
**Tom Feuchtel**, Univ. of Copenhagen  
**Alain Fischer**, INSERM  
**Wulfam Gerstner**, EPFL Lausanne  
**Karl-Heinz Glassmeier**, Inst. for Geophysics & Extraterrestrial Physics  
**Charles Godfrey**, Univ. of Oxford  
**Diane Griffin**, Johns Hopkins Bloomberg School of Public Health  
**Christian Haas**, Ludwig Maximilians Univ.  
**Steven Hahn**, First Hutchinson Cancer Research Center  
**Gregory J. Hanson**, Cold Spring Harbor Lab.  
**Niels Hansen**, Technical Univ. of Denmark  
**Dennis L. Hartmann**, Univ. of Washington  
**Chris Hawkesworth**, Univ. of St Andrews  
**Martin Heimann**, Max Planck Inst., Jena  
**James A. Hendler**, Rensselaer Polytechnic Inst.  
**Janet G. Hering**, Swiss Fed. Inst. of Aquatic Science & Technology  
**Ray Hilborn**, Univ. of Washington  
**Michael E. Himmel**, National Renewable Energy Lab.  
**Kei Hirose**, Tokyo Inst. of Technology  
**Ove Hoegh-Guldberg**, Univ. of Queensland  
**David Holden**, Imperial College  
**Laura Hooper**, UT Southwestern Medical Ctr at Dallas  
**Ronald R. Hoy**, Cornell Univ.  
**Jeffrey A. Hubbell**, EPFL Lausanne  
**Steven Jacobsen**, Univ. of California, Los Angeles  
**Peter Jonas**, Universität Freiburg  
**Barbara B. Kahn**, Harvard Medical School

**Daniel Kahne**, Harvard Univ.  
**Bernhard Keimer**, Max Planck Inst., Stuttgart  
**Robert Kingston**, Harvard Medical School  
**Hanna Kokko**, Univ. of Helsinki  
**Alberto R. Kornblith**, Univ. of Buenos Aires  
**Leonid Kruglyak**, Princeton Univ.  
**Lee Kump**, Penn State Univ.  
**Mitchell A. Lazar**, Univ. of Pennsylvania  
**David Lazer**, Harvard Univ.  
**Virginia Lee**, Univ. of Pennsylvania  
**Ottoline Leyser**, Univ. of New York  
**Olle Lindvall**, Univ. Hospital, Lund  
**Marcia C. Linn**, Univ. of California, Berkeley  
**John Lis**, Cornell Univ.  
**Richard Losick**, Harvard Univ.  
**Jonathan Losos**, Harvard Univ.  
**Ke Lu**, Chinese Acad. of Sciences  
**Laura Machesky**, CRUK Beatson Inst. for Cancer Research  
**Andrew P. Mackenzie**, Univ. of St Andrews  
**Anne Margolin**, Univ. of St Andrews  
**Oscar Marin**, CSIC & Univ. Miguel Hernández  
**Charles Marshall**, Univ. of California, Berkeley  
**Martin M. Matzuk**, Baylor College of Medicine  
**Graham Medley**, Univ. of Warwick  
**Virginia Miller**, UNC, Chapel Hill  
**Yasushi Miyashita**, Univ. of Tokyo  
**Richard Morris**, Univ. of Edinburgh  
**Edward Moser**, Norwegian Univ. of Science and Technology  
**Sam Navarro**, MRC Lab. of Molecular Biology  
**Naoto Nagasawa**, Univ. of Tokyo  
**James Nelson**, Stanford Univ. School of Med.  
**Timothy W. Nilsen**, Case Western Reserve Univ.  
**Pär Nordlund**, Karolinska Inst.  
**Helga Nowotny**, European Research Advisory Board  
**Stuart H. Orkin**, Dana-Farber Cancer Inst.  
**Christine Ortiz**, MIT  
**Elinor Ostrom**, Indiana Univ.  
**Andrew Oswald**, Univ. of Warwick  
**Jonathan T. Ouerckx**, Univ. of Arizona  
**P. David Pearson**, Univ. of California, Berkeley  
**John Pendry**, Imperial College  
**Reginald M. Penner**, Univ. of California, Irvine  
**John H. J. Petriani**, Memorial Sloan-Kettering Cancer Center  
**Simon Philpott**, Univ. of Florida  
**Philippe Poulin**, CNRS  
**Colin Renfrew**, Univ. of Cambridge  
**Trevor Robbins**, Univ. of Cambridge  
**Barbara A. Romanowicz**, Univ. of California, Berkeley

**Jens Rostrup-Nielsen**, Haldor Topsøe  
**Edward M. Rubin**, Lawrence Berkeley National Lab  
**Shimon Sakaguchi**, Kyoto Univ.  
**Michael I. Sanderson**, Univ. of Arizona  
**Jürgen Sandkühner**, Medical Univ. of Vienna  
**Randy Seeley**, Univ. of Cincinnati  
**Christine Seidman**, Harvard Medical School  
**Vladimir Shalae**, Purdue Univ.  
**Joseph Silk**, Univ. of Oxford  
**Montgomery Slatkin**, Univ. of California, Berkeley  
**Davor Solter**, Inst. of Medical Biology, Singapore  
**Allan C. Spradling**, Carnegie Institution of Washington  
**Jonathan Sprent**, Garvan Inst. of Medical Research  
**Elsbeth Stern**, ETH Zürich  
**Yoshiko Takahashi**, Wako Inst. of Science and Technology  
**Jürg Tschopp**, Univ. of Lausanne  
**Herbert Virgin**, Washington Univ.  
**Bert Vogelstein**, Johns Hopkins Univ.  
**Cynthia Volkert**, Univ. of Göttingen  
**Bruce D. Walker**, Harvard Medical School  
**Ian Walmsey**, Univ. of Oxford  
**Christopher A. Walsh**, Harvard Medical School  
**David A. Wardle**, Swedish Univ. of Agric Sciences  
**Colin Watts**, Univ. of Dundee  
**Detlef Weigel**, Max Planck Inst., Tübingen  
**Jonathan Weissman**, Univ. of California, San Francisco  
**Sue Wessler**, Univ. of Georgia  
**Ian A. Wilson**, The Scripps Res. Inst.  
**Timothy D. Wilson**, Univ. of Virginia  
**Xiaoliang Sunney Xie**, Harvard Univ.  
**John R. Yates III**, The Scripps Res. Inst.  
**Jan Zaanen**, Leiden Univ.  
**Mayana Zatz**, University of Sao Paulo  
**Jonathan Zehr**, Ocean Sciences  
**Huda Zoghbi**, Baylor College of Medicine  
**Maria Zuber**, MIT

## BOOK REVIEW BOARD

**John Aldrich**, Duke Univ.  
**David Bloom**, Harvard Univ.  
**Angela Croom**, Princeton Univ.  
**Richard Swedner**, Univ. of Chicago  
**Ed Wasserman**, DuPont  
**Lewis Wolpert**, Univ. College London



## Poop Scoop

New baby? Feeling like you're waist deep in dirty diapers? Forget diaper-collection services; just volunteer your infant for a poop study and researchers will take them off your hands for free. Dirty diapers, it seems, hold the key to measuring infant hormone levels.

Sex hormones, such as estrogen, are important for babies' healthy development. But some endocrinologists worry that children are exposed to too much additional estrogen via soy formula, plant fertilizers, and even plastics, which could cause faster-than-normal development and future problems with reproduction. However, few infants tolerate a frequent finger or heel prick, and so "very little is known about hormone levels in infants," explains Michelle Lampl, an anthropologist at Emory University in Atlanta.

Diapers, however, can be collected frequently and over a long period of time, perfect for a longitudinal study. Practicing on eight to 10 diapers collected from each of 32 largely breast-fed infants over 6 months, Lampl's group perfected a technique for extracting hormone levels from the poop, they reported online 11 November in *Frontiers in Systems Biology*.

They also perfected their diaper-collection technique. "It took years to find the right nappy and work out how you get the diaper fresh from the home to the lab," says Lampl. The secret: a cotton diaper, a Ziploc bag, and an ice pack.

## The Sound of Science

Two years ago, particle physicists at the European laboratory CERN near Geneva, Switzerland, rapped about the start-up of their enormous new atom smasher, the Large Hadron Collider (LHC). Now, they're taking it up a notch: On 6 December, scientists from the collaboration that takes data with LHC's ATLAS detector will be releasing a double album featuring everything from classical and medieval music to blues and heavy metal.

*Resonance* ([www.atlas-resonance.ch](http://www.atlas-resonance.ch)) will be released on homegrown label Neutralino Records (named after a hypothetical subatomic particle) and includes a song about the detector's workings by physicist Steven Goldfarb's Canettes Blues Band. In another tune, guitar band the TLAs (Three Letter Acronyms) laments that "when black holes destroy the Earth, I'll be in a meeting." Besides raising money for an orphanage in

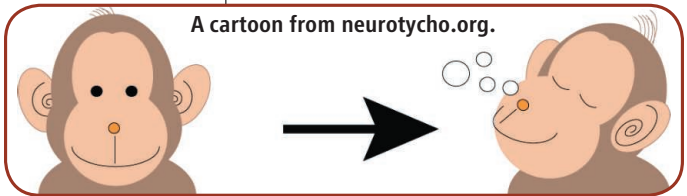
Nepal, Goldfarb hopes that the record will highlight how science, like music, is a creative enterprise. "We are not trying to compete with the Beatles or Motown," he says, "but to try and interest young people in physics." Imagine Paul McCartney's relief.

## Thanks for Sharing

The phrase "copyright-free repository of neuroscience data" doesn't exactly roll off the tongue. So when he decided to share his data, Naotaka Fujii of the RIKEN Brain Science Institute in Wako City, Japan, dubbed his collection "Neurotycho" (<http://neurotycho.org>). The name pays homage to the 16th century Danish astronomer Tycho Brahe, whose collected data inspired Johannes Kepler's laws of planetary

motion. Fujii's own data combines multi-electrode recording and motion capture to study adaptive behavior in primates.

Many animal researchers who put data online cloak it in technical jargon or hide it behind academic firewalls to forestall attacks by activists. Neurotycho, by contrast, highlights the information with simple explanations and cartoon illustrations. "I thought the benefit of starting Neurotycho was much larger than the risks," Fujii says. "We need new ideas from different fields for understanding neural mechanisms." The site's



A cartoon from neurotycho.org.

usefulness is catching on: In the first 10 days after it was launched last month, 150 neuroscience experts from around the world signed up.

## GOOOOOO SCIENCE!

It was just another Monday night for patrons gathered at a Philadelphia sports bar on 15 November to watch the Eagles play the Washington Redskins. And then a squad of cheerleaders burst through the door and, armed with megaphones and pompoms, explained football in terms of vector physics.

The Science Cheerleaders strike again. Like all superheroes, they lead double lives: All of them are either professional cheerleaders working on a scientific degree or former cheerleaders who switched to science-related careers. "I am very fortunate that I have the opportunity to fulfill both passions: science and dance," says Alyson, a Tennessee Titans cheerleader who plans to start medical school next year. (Like pro cheerleaders, squad members don't use their full names as a precaution against stalking.) In October, they performed at the U.S.A. Science & Engineering Festival on the National Mall in Washington, D.C. "Some people feel we are trying to improve the reputation of cheerleaders, while others feel this is helping scientists," says Darlene Cavalier, a former Philadelphia 76ers cheerleader who manages the Science Cheerleaders ([www.sciencecheerleader.com](http://www.sciencecheerleader.com)) and other science outreach programs. "I just hope it inspires as many people as possible!"



Bacteria that  
thrive on arsenic

1302

A gathering storm  
for U.S. universities

1304

## HIV/AIDS CLINICAL TRIALS

## A Powerful and Perplexing New HIV Prevention Tool

On 2 October, two dozen AIDS researchers gathered at the Eden Roc hotel on Millionaire's Row in Miami Beach, Florida, to learn whether an HIV prevention study they had just completed would become a millstone or a milestone for the field. In the six-country study, 1251 men and transgender women who have sex with men and were not infected with HIV at the study's start took an antiretroviral pill each day to see whether it could ward off infection with the virus. Another 1248 carefully matched participants took a placebo. Failure would cast a pall over the whole concept of oral pre-exposure prophylaxis (PrEP), which is now being tested in four other efficacy trials that involve 16,000 heterosexuals and injecting drug users (see table). Success would offer a powerful new option to dodge the virus—and might even

bottom line. "It was very, very dramatic," says Robert Grant, a virologist with the J. David Gladstone Institutes at the University of California, San Francisco (UCSF), who headed the trial. "People were overjoyed to be among one of the few prevention trials to have shown a protective effect."

*The New England Journal of Medicine* published a full report of the iPrEx results on 23 November. The global response was ecstatic, prompting no less than U.S. President Barack Obama to issue a statement. "I am encouraged by this announcement of groundbreaking research on HIV prevention," said Obama. "While more work is needed, these kinds of studies could mark the beginning of a new era in HIV prevention." But the good news was tempered by a dizzying array of complicated issues about

a treatment in AIDS drug cocktails, which means doctors can immediately start prescribing it "off-label" as a preventive.

Yet PrEP's role in public health remains anything but clear. "We don't think, 'Let's just sprinkle Truvada in the water supply and it solves the problem,'" says Kenneth Mayer, who headed an iPrEx study at Fenway Health in Boston, one of two U.S. sites. (Nine other trial sites were in Peru, Ecuador, South Africa, Brazil, and Thailand.) "It's an imperfect tool."

First, iPrEx shows only that the drug works in the specific high-risk population studied. Mayer and others stress that, as was done in the study, the drug should be offered as part of a prevention package that includes condom promotion and counseling. Experts worry that PrEP might lead people to take more risks than they would otherwise, offsetting the benefit of the pill, although this wasn't seen in the study.

PrEP could do harm, too, if it drove the evolution of Truvada-resistant HIV strains. When used as treatment, Truvada alone, versus in combination, is not strong enough

to keep the virus in check. Theoretically, if people did not know they were already HIV-infected and took Truvada, the virus could mutate around the drug. An increase in Truvada-resistant strains, which are already circulating at low levels, could undermine

both treatment and PrEP. Again, such drug resistance did not surface in iPrEx, but the researchers were rigorous in their efforts to exclude HIV-infected people and tested participants for new infections every 4 weeks.

For policymakers, the PrEP results raise difficult ethical and practical questions. Funding for HIV/AIDS is already insufficient: Ten million infected people who need treatment currently have no access to drugs. "I think it's going to be quite a while before we'd start using oral antiretrovirals for prevention," says epidemiologist Salim Abdool Karim of the University of KwaZulu-Natal in Durban, South Africa, which has more

### ONGOING PRE-EXPOSURE PROPHYLAXIS EFFICACY TRIALS >>>

| Trial                   | Location                                | Sponsor                         | Population                               | Intervention                                     | Expected Results |
|-------------------------|-----------------------------------------|---------------------------------|------------------------------------------|--------------------------------------------------|------------------|
| Bangkok Tenofovir Study | Thailand                                | U.S. CDC                        | 2400 injecting drug users                | Daily oral TDF                                   | 2012             |
| Partners PrEP           | Kenya, Uganda                           | Gates Foundation                | 4700 serodiscordant heterosexual couples | Daily oral TDF; daily oral TDF/FTC               | 2013             |
| VOICE                   | South Africa, Uganda, Zimbabwe          | Microbicide Trials Network, NIH | 5000 heterosexual women                  | Daily oral TDF; oral TDF/FTC; topical 1% TDF gel | 2013             |
| FEM-PrEP                | Kenya, South Africa, Tanzania, Zimbabwe | FHI, USAID, Gates Foundation    | 3900 heterosexual women                  | Daily oral TDF/FTC                               | 2013             |

TDF = tenofovir; FTC = emtricitabine

**More news soon.** Large PrEP studies in different populations will start reporting results by 2012.

slow HIV's spread in entire communities.

Unlike the many HIV prevention trials that have failed or had positive but barely significant results, the study—called the Pre-Exposure Prophylaxis Initiative (iPrEx)—showed unequivocally that the treated group had 44% fewer infections after an average of 1.2 years. More encouraging still, most of the failure seemed to occur among those who did not take the pill as directed: A small substudy found that risk of infection plummeted by 92% in people who had measurable drug levels in their blood. The researchers applauded and some even cried when they heard the

human behavior, ethics, resources, risk, and public health.

The iPrEx study, which cost \$43.6 million and was conducted between July 2007 and December 2009, has one clear-cut message: A pill can dramatically lower the chances of transmission of HIV through receptive anal intercourse in men who have many partners—on average, 18 in the 12 weeks preceding the start of the trial—and frequently do not use condoms. The pill, Truvada, made by Gilead Sciences in Foster City, California, combines two anti-HIV drugs, tenofovir and emtricitabine. Truvada is already on the market and widely used as





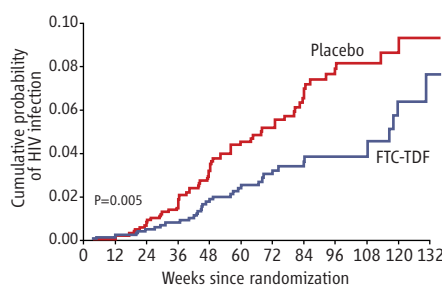
infected people than any country.

In wealthy countries, no one knows whether insurance companies will cover the use of Truvada as a preventive, and there are no guidelines for prescribing PrEP, although in the next few weeks, the U.S. Centers for Disease Control and Prevention says it will publish interim guidance.

Anthony Fauci, director of the U.S. National Institute of Allergy and Infectious Diseases (NIAID), which paid for two-thirds of iPrEx (the Bill and Melinda Gates Foundation picked up the balance), says Truvada's success as PrEP could have far-reaching impact on other HIV prevention studies. Ethical principles demand that researchers minimize risk for people who participate in studies, which means offering known effective interventions in the control arms of trials of vaccines or other PrEP compounds. Fauci says NIAID will now review "virtually every study that we have ongoing or planned" to assess whether trials that have placebo controls should instead offer Truvada. "It's going to create welcome work for everyone in HIV prevention science," says Jeremy Sugarman, a bioethicist at the Johns Hopkins Bloomberg School of Public Health in Baltimore, Maryland. Sugarman, who chairs the ethics working group of the NIAID-funded HIV Prevention Trials Network, stresses that he's thrilled about the positive results. "If they make our ethical questions harder to answer, so be it."

UCSF's Grant contends that the iPrEx results create new opportunities that might mitigate some of the potential downsides. First, he wonders whether real-world adherence might be better than in a clinical trial setting, as people know that they are taking an active drug and that it works. Adherence also could differ in different populations. Epidemiologist Connie Celum of the University of Washington, Seattle, is currently heading a PrEP study in 4700 Kenyan and Ugandan heterosexual couples in which only one partner is known to be infected. The Partners PrEP Study carefully monitors drug adherence, and preliminary evidence suggests it is much higher than in iPrEx, which Celum suspects is because the uninfected long-term partners are highly motivated.

As for resource limitations sidelining PrEP, prophylactic use of Truvada could create more competition and lead to a reduc-



**Fielding questions.** Robert Grant (right) spoke about potential risks and benefits with communities in Peru before launching iPrEx. Positive results (left) mean clinicians everywhere must do the same.

Ultimately, PrEP's popularity may be tied to whether Gilead asks the U.S. Food and Drug Administration (FDA) for a label change to include its use as a preventive. Although not required, insurers will often reimburse only for indications approved by regulatory bodies, and many countries follow the lead of FDA. Gilead says it wants to have "frank" talks with FDA and other stakeholders before it decides to seek licensure for Truvada as a preventive. "We'll have, I imagine, a very interesting discussion about the potential risks and benefits associated with this kind of a modality, and I think that will govern what we choose to do," says Howard Jaffe, president of the Gilead Foundation, a nonprofit started by the company to help poor communities combat HIV and hepatitis B and C.

Confusing as PrEP's fate might seem, Fenway Health's Mayer stresses that the iPrEx results are an important milestone for the failure-weary HIV prevention field. "This plus CAPRISA means we've crossed the Rubicon," says Mayer. "Antiviral chemoprevention works, no question."

—JON COHEN

tion in the price of the drug, which ranges from \$11 per month for a generic version sold in poor countries versus up to nearly \$1000 per month for the retail Gilead product. And PrEP costs would fall if people do not need to take it every day: Truvada has a long half-life, and ongoing and future studies will assess whether it will work with less frequent dosing. CAPRISA 004, a PrEP trial with a vaginal microbicide that contained tenofovir, revealed in July that inserting the gel before and after sex alone cut transmission by 39%.

In keeping with a push to link prevention and treatment—treated people are likely less infectious—PrEP might encourage more people to undergo HIV tests. "No one is getting tested hoping to be positive and start treatment," says Grant. "People are hoping to be negative. This becomes a potent motivator to get a test."



NEWSMAKER INTERVIEW: MICHEL SIDIBÉ

# New HIV Infections Drop, But Treatment Demands Rise

Two years ago on World AIDS Day, 1 December, Michel Sidibé was appointed the new executive director of the Joint United Nations Programme on HIV/AIDS (UNAIDS). An economist by training and a native of Mali, Sidibé came into the job when funding for HIV/AIDS was still flush. But the global economic crisis that fall threatened to end the era of extraordinary largesse, which enabled many poor countries to rapidly increase the number of people who were receiving antiretroviral treatment. On World AIDS Day today, that rapid growth has come to a standstill, and Sidibé is wrestling to renew the momentum and turn the funding situation around.

In its new 359-page *Global Report* on the epidemic, UNAIDS spells out the challenges in detail, documenting the country-by-country prevalence that adds up to an estimated 33.3 million HIV infections. The report, as usual, is a sweet-and-sour dish, emphasizing that despite increasing concern about funding, 33 countries—two-thirds of them in sub-Saharan Africa—have seen rates of new HIV infections drop by more than 25% since 2001. Sidibé spoke with *Science* from New York City, where he was visiting the U.N. headquarters. —JON COHEN

**Q: What's the main news from the report?**

**M.S.:** For me the most exciting news is we have broken the trajectory of the AIDS epidemic. It's the first time through data and analysis we can show that 56 countries have stabilized or significantly slowed the rate of new HIV infections.

**Q: How confident are you that these stabilizations are due to prevention efforts as opposed to HIV having infected the most vulnerable people years ago and saturating populations?**



**M.S.:** I think it's mainly due to prevention combined with treatment. To give you a good example, in 59 countries, fewer than 25% of the men reported having sex with more than one partner in the last 12 months, which is spectacular. A few years ago we were seeing the opposite trend. Condom use and availability have also increased significantly. And young people are leading the prevention effort by delaying the first time they have sex. I don't think it's just saturation.

**Q: The new report has scorecards that summarize the prevention, treatment, funding, and human rights situations in each country. It doesn't look like everyone is achieving what you want.**

**M.S.:** I tried to introduce this scorecard concept to better analyze the gaps. We're closing the gap between treatment and prevention. Just 2 years ago, for every two people we were putting on treatment we were having five new infections. Now it's one to two. That's a very good signal.

**Q: What do you think of regions that aren't stabilized, like Eastern Europe and Central Asia, which saw incidence increase by 25% between 2001 and 2009?**

**M.S.:** In these regions, prevention services for drug users are below 35%, which is far short of what's needed. If we have an epidemic mainly driven by drug use and we don't have prevention services available, it will be impossible to see the reduction we're seeing in other parts of the world. In Bangladesh and Indonesia, we're having difficulty reaching the sex worker and the client with HIV prevention services. It will also be a question of how we deal with laws and stigma and prejudice that are making people not have access to the services.

**Q: Where have you seen success that you didn't expect?**

**M.S.:** I was visiting Iran and was very surprised to see one of the most progressive programs in a prison setting where they were having condom distribution and methadone maintenance for prisoners.

**Q: What do you think of the 1-year retention rate of people on antiretrovirals? It's 95% overall but falls to 47% in one country, and after 2 or 3 years it plummets in many places.**

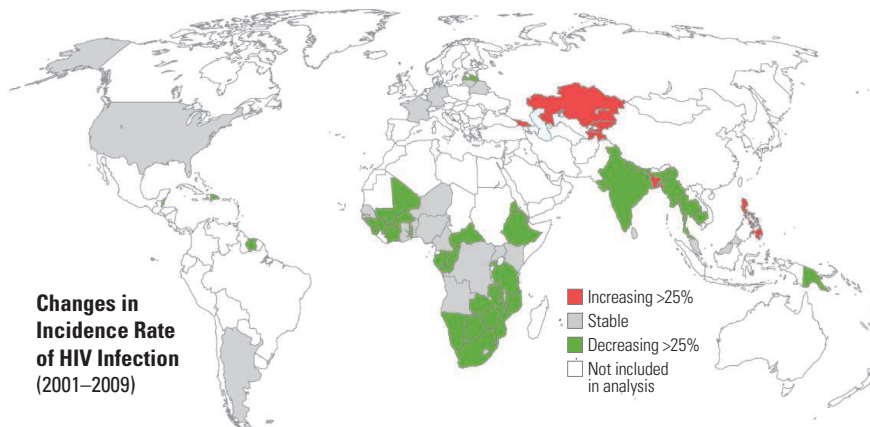
**M.S.:** We need a serious monitoring of the dropout rate. Dropouts have become one of the critical issues to deal with because we're going to have increasing drug resistance and a more complex problem to deal with.

**Q: On human rights, are we making progress or sliding backward? Are you concerned about the homophobic laws being pushed in Uganda?**

**M.S.:** Without any doubt, Uganda is one of the model countries for us. It's one of the first countries to break the conspiracy of silence, putting people with AIDS at the front line. I raised this issue with Uganda's government, and I certainly hope they will take the decision that is most appropriate for protecting those people.

**Q: Do you think we're at a point where we cannot sustain people who have already started ARVs?**

**M.S.:** We're at that point, and it's very, very scary. Most of the programs will have difficulty increasing the number of people on treatment. Most just are trying to maintain what already is being implemented.



CREDITS (TOP TO BOTTOM): J. COHEN/SCIENCE; UNAIDS

# What Poison? Bacterium Uses Arsenic To Build DNA and Other Molecules

From elephants to the bacterium *Escherichia coli*, all forms of life on Earth depend on the same six elements: oxygen, carbon, hydrogen, nitrogen, phosphorus, and sulfur. “The paradigm is that the chemistry of life is so specific that any change in chemistry also changes molecular stability and reactivity, which would not be tolerated,” says Clara Chan, a geomicrobiologist at the University of Delaware, Newark.

In a paper published online by *Science* ([www.sciencemag.org/cgi/content/abstract/science.1197258](http://www.sciencemag.org/cgi/content/abstract/science.1197258)) this week, however, an exception to that rule makes a surprising debut. Meet GFAJ-1, a bacterial strain that researchers say can replace the phosphorus in its key biomolecules, including DNA, with the legendary poison arsenic. “This is a very impressive and exciting discovery,” says Barry Rosen, a biochemist at Florida Inter-

national University in Miami. “The implication of this work is that life can be quite different from what we know,” agrees Chan.

Others were skeptical. The arsenic-containing compound arsenate is much more unstable than phosphate in water, and no cell would be able to cope with that, critics argued. To test her hypothesis, Wolfe-Simon collected mud from Mono Lake, California, a desert body of water known for having high arsenic levels, and grew the microorganisms from it in increasing concentrations of arsenate. She didn’t add any phosphate or other phosphorus-containing compounds to the growth medium, as is typically done to sustain microbes. Instead, she periodically transferred the growing cultures to a new dish to reduce the concentration of any original phosphorus to the point that any microbe making new DNA or other biomolecules would need to use the arsenic to survive.

Like others, says Wolfe-Simon, she didn’t really expect to find any survivors. So she was

forming each fraction. They also separated out the DNA from the bacteria and analyzed its composition using a technique called high-resolution secondary ion mass spectrometry; the isolated DNA contained arsenic.

Tests utilizing the intense x-rays at a synchrotron facility offered additional support, indicating that at least some of the arsenic in the bacteria was in the form of arsenate with the appropriate molecular bonds to carbon and oxygen atoms to replace the phosphates in DNA and other molecules.

Such work has convinced many that Wolfe-Simon’s team has isolated a bacterium that uses arsenic to grow. “The organization of the experiments presents convincing and exhaustive results,” says Milva Pepi, an environmental microbiologist at the University of Siena in Italy. But not everyone agrees. Rosen finds the study “believable” but says he still has lingering concerns that the arsenic is simply concentrated in the bacterial cell’s extensive vacuoles and not incorporated into its biochemistry. He would like to see Wolfe-Simon’s team demonstrate a functional arsenic-containing enzyme, for example. Steven Benner, an astrobiologist at the Foundation for Applied Molecular Evolution in Gainesville, Florida, is more skeptical: That GFAJ-1 uses arsenic as a replacement for phosphorus, “is, in my opinion, not established by this work,” he says.

Wolfe-Simon isn’t arguing that GFAJ-1 prefers, or even naturally uses, arsenic. Mono Lake has a lot of phosphorus as well as arsenic, and the strain grows better when supplied with phosphorus. But to her and others, GFAJ-1 is proof that phosphorus-free life forms can exist and may do so somewhere on Earth. Next, Wolfe-Simon wants to collect samples from places with high arsenic but low phosphorus concentrations in hopes of finding microbes that depend solely on the former.

Wolfe-Simon speculates that organisms like GFAJ-1 could have thrived in the arsenic-laden hydrothermal vent-like environments of early Earth, where some researchers think life first arose, and that later organisms may have adapted to using phosphorus. Others say they’ll refrain from such speculation until they see more evidence of GFAJ-1’s taste for arsenic and understand how the DNA and other biomolecules can still function with the element incorporated. “As in this type of game changer, some people will rightly want more proof,” says microbiologist Robert Gunsalus of the University of California, Los Angeles. “There is much to do in order to firmly put this microbe on the biological map.”

—ELIZABETH PENNISI



**Search for unusual life.** Felisa Wolfe-Simon collects samples from Mono Lake (left), where high arsenic levels proved conducive to the evolution of arsenic-using microbes.

thrilled and surprised when one evening she checked the latest cultures under the microscope and saw fast-moving bacteria. She rechecked the components of the culture media to confirm there were no phosphorus contaminants. She and her colleagues then began to subject the microbes to sophisticated analyses to see if arsenic had been utilized by the bacteria. “I held my breath with every one,” says Wolfe-Simon.

One form of mass spectrometry showed that the arsenic was inside the bacterial cells and not some impurity sticking to the outside of the cell. When the researchers added radioactively labeled arsenate to the bacteria’s culture, they were later able to discern its presence in the protein, lipid, nucleic acid, and metabolite fractions of the cells, suggesting that arsenic had been incorporated in molecules

Despite that, Wolfe-Simon speculated that some microbes might be able to adapt to using

arsenic. Others were skeptical. The arsenic-containing compound arsenate is much more unstable than phosphate in water, and no cell would be able to cope with that, critics argued. To test her hypothesis, Wolfe-Simon collected mud from Mono Lake, California, a desert body of water known for having high arsenic levels, and grew the microorganisms from it in increasing concentrations of arsenate. She didn’t add any phosphate or other phosphorus-containing compounds to the growth medium, as is typically done to sustain microbes. Instead, she periodically transferred the growing cultures to a new dish to reduce the concentration of any original phosphorus to the point that any microbe making new DNA or other biomolecules would need to use the arsenic to survive.

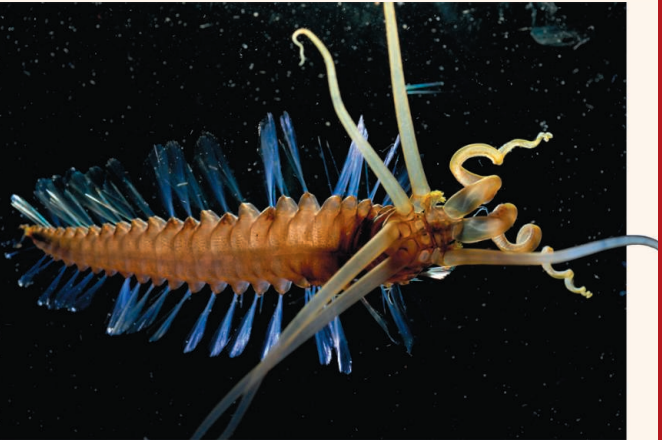
Like others, says Wolfe-Simon, she didn’t really expect to find any survivors. So she was



## From Science's Online Daily News Site

### Meet the Squidworm

Deep in the Pacific Ocean, scientists have discovered a remarkable new creature that they have dubbed the "squidworm." Karen Osborn, then at the Scripps Institution of Oceanography in San Diego, California, and colleagues used a remotely operated vehicle to find *Teuthidodrilus samae* at the bottom of the Celebes Sea off the eastern coast of Borneo. As the team reports online in *Biology Letters*, the segmented worm sports a series of 10 large appendages near its head that give it a squidlike appearance. <http://scim.ag/squidworm>



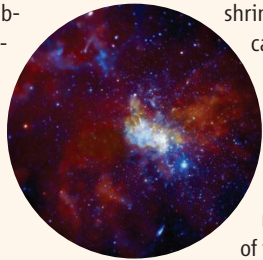
### Black Hole May Offer Clues To Extra Dimensions

String theory posits that space has more than three dimensions, curled up in loops so small that they could probably be probed only in high-energy particle collisions. Now one theorist suggests these hypothesized extra dimensions might reveal themselves in the stars.

According to Einstein's theory of gravity, matter warps spacetime, changing the paths of free-falling objects. It even affects light; when light from a star passes near a galaxy, its path bends, changing where the star appears in the sky—an effect called gravitational lensing.

Now, physicist Amitai Bin-Nun of the University of Pennsylvania argues in *Physical Review D* that gravitational lensing around the supermassive black hole thought to be at the center of the Milky Way might provide a way to search for extra dimensions. Bin-Nun used numerical simulations to show that, in a world with extra dimensions compared with one without, a star known as S2 could be as much as 44% brighter when it reaches its peak brightness in 2018.

But researchers say the hypothesis will be hard to validate with telescopes, and if the black hole is rotating, it would produce effects that could easily be confused with Bin-Nun's predictions. <http://scim.ag/extra-dimensions>



team has used genetic engineering to accomplish something similarly curious in mice.

Scientists don't fully understand what triggers aging, but many suspect the gradual shrinking of telomeres, the protective DNA caps on the end of chromosomes. The telomeres shrink as cells divide, and telomerase, the enzyme that maintains the caps, isn't typically active in adult tissues.

To investigate the connection, molecular biologist Ronald DePinho of the Belfer Institute at the Dana-Farber Cancer Institute of Harvard Medical School and colleagues first genetically engineered mice to lack a working copy of the telomerase gene. The animals aged prematurely and died at about

6 months—mice usually live for 3 years. Then the team created a new batch of mice with the same infirmity but with a telomerase gene activated by a certain drug. The researchers let these mice prematurely age, then at 6 months they switched on the telomerase gene.

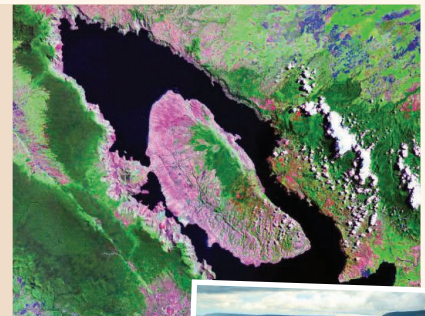
The burst of telomerase production spurred almost total recovery, the researchers report online in *Nature*. This indicates that the cells that divide to replenish tissues don't simply die when their telomere clock expires, but can be revived, says DePinho. Other researchers point out that these mice were genetically modified, so it still remains to be seen if aging can be delayed in a normal mouse—or in a human.

<http://scim.ag/aging-mouse>

### Giant Eruption Cut Down to Size

Just how devastating was the eruption of the Indonesian "supervolcano" Toba 74,000 years ago? Some researchers have suggested that it locked the world in a deep freeze, whereas others have put the cooling at only 3° to 5°C. Similarly, some archeologists have proposed that the explosion impacted human evolution, creating a genetic "bottle-neck" where only the survivors' genes got passed on; others have argued that people would have survived the eruption handily.

Now researchers led by climate modeler Claudia Timmreck of the Max Planck Institute for Meteorology in Hamburg, Germany, have weighed in with a climate model that focused specifically on the way the blast's sulfate aerosol particles cooled the atmosphere by reflecting the sun's rays. Assuming that Toba pumped out about 850 million metric tons of sulfur, the number used in previous models and supported by ice core data, temperatures would have dipped only 3° to 5°C across the globe, the team reports online in *Geophysical Research Letters*. Overall, Toba didn't wipe out flora and fauna, Timmreck says, but it would have made life harder for a few years. <http://scim.ag/toba-impact>



Toba's crater, now a lake (inset).



### The Curious Case of the Backwardly Aging Mouse

In F. Scott Fitzgerald's short story, "The Curious Case of Benjamin Button," an old man gets younger with each passing day. Now a

Read the full postings, comments, and more at <http://news.sciencemag.org/sciencenow>.

## U.S. RESEARCH UNIVERSITIES

# Panel Explores New Funding Pact With Washington

The leaders of U.S. higher education have complained for a decade or longer that the unwritten arrangement between the federal government and the universities, struck in the 1950s, is badly strained. According to that arrangement, the government would pay the full cost of university research it funds and, in return, universities would educate the next generation of scientists. Over time, however, a growing number of federal regulations and a cap on reimbursement rates have led to billions in university research costs that aren't reimbursed. Rising federal spending has helped keep the relationship intact, but now a fiscal crisis threatens to rip it apart.

That was the backdrop to an intense meeting last week at the National Academies in Washington, D.C. A blue-ribbon panel asked by Congress to recommend how the coun-

inherent conservatism within academia. His telephoned comments struck a chord with the panel, which also welcomed Alexander's suggestion that they compile separate rankings of "10 things that cost money and 10 things that don't."

"If you assume that the amount of research funding is fixed, then the only question is how it's divvied up," says Chad Holliday, chair of the panel. "So I hope that we can come up with a robust set of recommendations, some of which do not require more money."

The panel's charge deliberately mimics the 2005 request from Congress that led to *Rising Above the Gathering Storm*, the academies' improbably influential report on how the overall U.S. scientific enterprise could strengthen the economy. The higher education community, which planted the seed

for the congressional request, hopes the new report will address what it sees as glaring flaws in the system that governs the \$32 billion a year that the federal government now spends on academic research.

At the top of the list, university leaders want changes in the formula used to reimburse universities for the ancillary costs of conducting federally funded research on campus.

The negotiated reimbursement rate is too low and allows too many exceptions, they say. As a result, universities must dip into their own coffers or curtail other educational activities. "We fail to recover \$600 million a year," says Steven Beckwith, vice president for research for the 10-campus University of California system, tallying up the shortfall caused by a cap in so-called indirect cost recovery rates and by various exceptions to those rates.

University leaders also want the government to ease some of the administrative requirements that come with federal research dollars, saying they drive up costs without providing any significant societal benefits or protections. "When I began I wasn't sure about that," says Holliday, a former DuPont CEO. "But after our discussion, I now see

how those rules put an undue burden on universities and force them to make spending choices they shouldn't have to make."

The gloomy fiscal outlook led panel member James Duderstadt to propose a bold quid pro quo in line with Alexander's description of the problem. What if, he said, the community abandoned its perennial request for more—on the grounds that investing in research is a public good that will pay large dividends—and accepted flat funding in return for the government allowing universities to be reimbursed fully for what they consider the true cost of hosting federally funded research? He also suggested an easing of the regulatory burden, which he speculated might save enough in administrative costs to nearly eliminate the shortfall in paying for the so-called indirect costs of research: administration, buildings, utilities, etc.

Duderstadt says his idea is just a trial balloon to address a problem "that we've spent 40 years debating and haven't made a dent in solving." But Robert Berdahl, president of the Association of American Universities, an organization of 63 research powerhouses, joined many in the room in voicing support for it. "I don't think we'll see an increase in the federal investment in research in the foreseeable future so, yes, I think we'll have to redistribute the money in a different way," Berdahl said. "I would support an increase in funding at the inflation rate," he added, a rise much flatter than the 7- to 10-year doubling that the community has long sought.

At the same time, Duderstadt acknowledged that increasing reimbursement for overhead costs, in a flat budget, would leave agencies with less money for research grants. That would increase competition among faculty members already bemoaning low success rates on their applications. "Would your faculty support that approach?" wondered panelist Walter Massey, president of Morehouse College in Atlanta and a former director of the National Science Foundation. "They want a grant with lower, not higher, indirect costs. Could you bring them around?"

Several university administrators thought they could, provided the changes were explained properly. "The challenge is to be consistent and understandable," said Kim Wilcox, provost and vice president for academic affairs at Michigan State University in East Lansing. "If we can do that, then it's up to us to explain it to the faculty."

—JEFFREY MERVIS



**Cut-rate science?** Federal grants don't cover the true cost of academic research, say university administrators.

try's research universities can bolster the nation's well-being (*Science*, 9 July, p. 126) spent a day and a half in public discussion with several dozen leaders in higher education, followed by a day of private deliberations aimed at producing a final report next summer. Overshadowing the discussions was growing concern that federal investment in academic research may shrink as the government struggles to reduce the federal deficit, even as states are cutting support to their flagship public research institutions in an attempt to balance their recession-battered budgets.

Senator Lamar Alexander (R-TN), one of four science-savvy legislators from both parties who requested the study, summed up the community's predicament this way: too many rules, not enough money, and an



## U.S. ENERGY POLICY

# With Money Tight, White House Panel Offers New Path to Energy Research

In a period of avowed austerity in Washington, D.C., can the government spark a technological revolution in energy without raiding the U.S. Treasury? A new report embraced by the White House says yes. Small surcharges on energy production and use could raise the billions of dollars needed for additional research and development, it says, and a quadrennial energy review and other administrative changes would help spend the money wisely. But even fans of the plan, which echoes other recent high-level reports on the topic, say it faces sizable political and logistical challenges.

"American economic competitiveness, environmental stewardship, and enhanced security depend on picking up the pace of energy technology innovation," says the report, *Accelerating the Pace of Change in Energy Technologies Through an Integrated Federal Energy Policy*, from the President's Council of Advisors on Science and Technology (PCAST). It notes that "extraordinary actions" by the government are needed to do so.

The report says that current federal energy R&D spending should be tripled, to \$16 billion a year. Its innovative approach to obtaining the needed funds is likely to make waves. Running for president in 2008, Barack Obama proposed a 10-year, \$150 billion fund for energy funded mostly by revenue from a national greenhouse gas emissions trading scheme. Even months before that scheme was declared dead in Washington, PCAST member Maxine Savitz told *Science*, members looked at other sources, in particular surcharges on energy transmission or partnerships with industry. A one-tenth of a cent surcharge for every 1 kWh of electricity used, for example, could generate \$4 billion, the report says, as could a 2-cent-a-gallon charge on liquid fuel.

But the government needs a "more coordinated and robust" effort to spend that money well, the report declares. Toward that end, PCAST called for a "Quadrennial Energy Review (QER)," led by the Department of Energy and involving all relevant federal agencies, industry, and the public. The QER "could reduce bureaucracy" by getting federal agencies "reading from the same memo," says Robert Simon, staff director for the Senate Energy and Natural Resources Committee, who gave input to PCAST members before



Fill 'er up. A 2-cent-per-gallon fee on liquid fuels could help fund a tripling of spending on energy R&D, a study says.

the report was written. The review could be a useful process for defining and coordinating energy policy, agreed David Goldston of the Natural Resources Defense Council in Washington, D.C., as long as it can avoid reaching bland conclusions or drawing a "map as big as the territory" it is examining.

At the same time, warned Goldston, a former staff director of the House of Representatives science committee, collecting "revenue streams" for innovation from various energy sources could create a "patchwork of separate energy policies." Political or legal entanglements can stymie surcharge systems, Simon added, pointing to a \$23 billion fund for nuclear waste storage, funded by small charges on electricity produced by nuclear power, that's never been spent on storing fuel.

Despite those challenges, the report's backers hope that the message in its 39 pages will find a receptive audience among those who feel that action is urgently needed. By coincidence, Energy Secretary Steven Chu, who requested the report, spoke about the need for energy innovation during a previously scheduled talk to the national media only 2 hours after the report's release. "America still has the opportunity to lead in a world that needs a new energy revolution," he said. "But I think time is running out."

—ELI KINTISCH

## ScienceInsider

### From the *Science* Policy Blog



The Howard Hughes Medical Institute is holding a competition to support the research programs of **early-career scientists trained in the United States who have now returned home**. Up to 35 scientists in 18 countries will receive \$650,000 over 5 years. The deadline is 23 February. <http://scim.ag/Hughes-international>

**Two car bombings in Tehran** killed one Iranian nuclear scientist and wounded a second in circumstances similar to the slaying of a theoretical physicist in January. Government leaders accuse the West of trying to dismantle Iran's nuclear program. <http://scim.ag/iranian-bombings>

President Barack Obama has asked his bioethics council to conduct a "thorough review" of **U.S. rules protecting human subjects** to ensure that they "protect people from harm or unethical treatment, domestically as well as internationally." <http://scim.ag/human-subjects>

An expert panel says the Dutch government failed to mount a robust response to the world's **worst outbreak of Q fever**, a disease transmitted from farm animals to humans that has killed 14 people and sickened thousands more since the outbreak began in 2007. <http://scim.ag/Q-fever-response>

University officials say it's not clear that **new immigration policies in the United Kingdom** will do more to allow the free flow of foreign scientists into the country. <http://scim.ag/uk-visa-policies>

The Croatian government has backtracked on a plan to store all of the country's **low- and medium-level nuclear waste** at a major research institute in the heart of its capital. <http://scim.ag/zagreb-waste-reversal>

Germany's high court has upheld a **law governing genetically modified crops** that requires a buffer zone and a public database of planting, and that holds researchers who plant GM crops liable for any pollen that escapes. <http://scim.ag/german-gm-law-upheld>

For more science policy news, visit <http://news.sciencemag.org/scienceinsider>.





# Brazilian Science: Riding a Gusher

**A fast-growing economy and oil discoveries are propelling Brazil's research to new heights. But scientific leaders must overcome a weak education system and a low-impact track record**

**NATAL**—Miguel Nicolelis stands with his arms spread, pointing out a rectangular pit carved into dry earth on the outskirts of the Brazilian beach city of Natal. “That is where the supercomputer will go,” he says. And, pointing to an area still thick with shrubbery, “that is the sports complex.”

Nicolelis is Brazil's best-known scientist. A neurobiologist at Duke University in Durham, North Carolina, he is renowned for spectacular experiments that use signals tapped from the brains of monkeys to make robots walk. But when Nicolelis launched plans in 2003 for a neuroscience institute in Brazil's backward northeast districts, few thought it could work (*Science*, 20 February 2004, p. 1131).

The notion was to pair cutting-edge science with a social mission: developing one of

Brazil's poorest regions. Nicolelis, who now spends part of the year in Brazil, is eager to offer a visitor “categorical proof” of success. He's built two hands-on science schools for children and a maternity clinic, and recruited 11 Ph.D. neuroscientists who run labs in a temporary headquarters. Within a few months, he says, \$25 million in Brazilian federal money should begin pouring into his sandy acreage, creating the sprawling neuroscience complex Nicolelis calls his “Campus of the Brain.”

“In Brazil we need science to build a country,” says Nicolelis, an energetic nationalist whose passions include wearing a green Palmeiras soccer club hat and downing pitchers of yellow maracujá juice. “This

place is going to create the next generation of Brazilian leaders.”

Some continue to think Nicolelis's idea is eccentric. But his timing couldn't have been better. Over the past 8 years, Latin America's largest nation has begun to boom. Its economy is growing fast, and it has become a player in world affairs, reveling in an unprecedented bout of self-confidence. It will host the World Cup in 2014 and the Olympics 2 years later.

The good times are lifting science, too. Between 1997 and 2007 the number of Brazilian papers in indexed, peer-reviewed journals more than doubled to 19,000 a year. Brazil now ranks 13th in publications, according to Thomson Reuters, having surpassed the Netherlands, Israel, and Switzerland. Brazil's universities awarded twice as many Ph.D.s this year as they did in 2001, and thousands of new academic jobs have opened up on 134 new federal campuses.

It's a reversal of fortune for a nation that during the 1990s was beset by dire economic problems. Back then, researchers scrounged for funds; Brazil even saw its flag removed from the logo of the International Space Station after it failed to come up with funding

**Online**  
**sciencemag.org**  
Podcast interview  
with author  
Antonio Regalado.



**Proud moment.** Neuroscientist Miguel Nicolelis at the foundations of his “Campus of the Brain” in northeastern Brazil.



**Science hub.** Brazil's richest city, São Paulo, and eponymous state are home to most of the nation's research.

to build six components. "We kept thinking smaller and smaller," says Sérgio Rezende, science minister for the past 5 years. "If we couldn't solve small problems, how could we solve big ones? Now we are in a position to think big again."

The fuel that propels science in Brazil is an R&D tax on big industries; it has swelled the budget of Rezende's ministry to \$4 billion, up from \$600 million a decade ago. The national oil company, Petrobras, is the largest contributor (see sidebar, p. 1308). Brazil restarted its nuclear research program in 2008, after a 20-year lull, and in October, a delegation traveled to Geneva to negotiate associate membership in CERN. With Brazil's economy growing at a pace of 7% this year, the country can afford the dues of \$14 million per year.

Scientists here say their arguments in favor of more education, innovation, and technology have been heard in Brasília, the capital, and they expect budgets to keep growing under Brazil's president-elect, Dilma Rousseff, the first woman to hold that post. By 2020, say officials of the Brazilian Society for the Advancement of Science, Brazil should again redouble or triple output of students, papers, and spending and become a "formidable" force in science. Government officials want to see Brazil among the top 10 science nations.

But Brazil is not formidable yet. Like Nicolelis's institute—where construction is several years behind schedule—Brazil's scientific output trails its ambitions. The country produces few high-impact papers and



## Highlights

**Amazon:** Half as large as Europe, the Brazilian Amazon is home to fewer than 3000 Ph.D. scientists. Government incentives seek to increase Brazil's scientific presence in the forest region.

**Brasília:** Brazil's rapid economic growth means rising budgets at the Ministry of Science and Technology. It now spends about \$4 billion a year.

**Natal:** Construction is beginning on a \$25 million "Campus of the Brain" conceived by Miguel Nicolelis, a neuroscientist at Duke University.

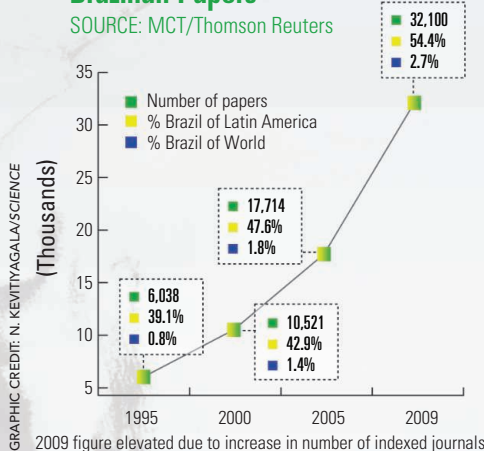
**Rio de Janeiro:** A massive new R&D facility built by oil company Petrobras is billed as the largest laboratory in the Southern Hemisphere. Workers will explore deep-water oil deposits.

**São Paulo:** The wealthiest state in Brazil is also its science leader. São Paulo researchers publish half of all Brazilian papers.

## Brazilian Science Begins to Boom

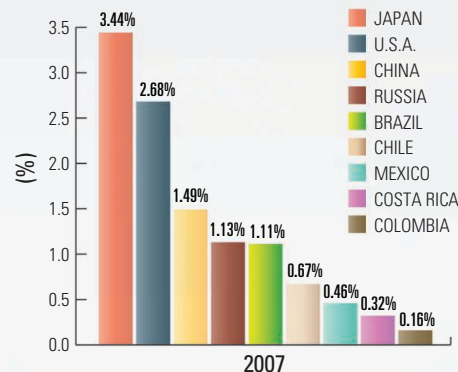
### Brazilian Papers

SOURCE: MCT/Thomson Reuters



### R&D Spending as % of GDP

SOURCE: IADB



### U.S. Patents for Selected Countries

SOURCE: USPTO





only a trickle of patents. Its primary and secondary public education system is in shambles, leaving the nation of 195 million chronically short of technical workers. “We need to be lucid and not fall into a victory discourse,” cautions Sidarta Ribeiro, a neuroscientist trained at Rockefeller University in New York City and co-founder of Nicolelis’s brain institute. “In terms of impact we are marginal. The external discourse for the world should be that we are interested in science and we are growing. The discourse internally should be, ‘Let’s improve. Let’s focus on merit.’”

### Boom times

Brazil is clearly breaking away from the pack in Latin America, indicators show. Brazil now accounts for over 60% of all research spending in Latin America, and Brazilian scientists write half of the papers. Brazil’s science bureaucracy is influential, too, having had its own ministry since 1985. That’s a step Argen-



**Tweaking nature.** Researcher in a government research lab in Brasília inspects a transgenic cotton plant.

tina took only 3 years ago and which neighbor Bolivia is still debating. “Brazil is the only example in Latin America where 1% of GDP goes into R&D and the science minister is a physicist that still publishes. So Brazil is the beacon,” says Juan Asenjo, president of the Chilean Academy of Sciences.

Globalization of markets is also working in Brazil’s favor. Like other Latin Ameri-

can countries, Brazil’s research base is heavily skewed toward agriculture, ecology, and infectious diseases—it is first in the world in publications related to sugar, coffee, and orange juice. Brazil’s cattle industry produces 33% of the world’s cow embryos. Once a sideshow, such research is increasingly well placed to address global preoccupations over food production, climate change, and conservation.

Nicolelis says he sees an “emerging tropical way of doing science” driven by research on renewable energy, agriculture, water, and animal and plant genetics. “These are the issues defining the planet, and, believe it or not, the players are down here,” says Nicolelis.

Biological research is a hot area of growth. The Empresa Brasileira de Pesquisa Agropecuária, the government-owned agricultural research company known as Embrapa, plans to hire 700 new researchers this year. Embrapa is considered one of the world’s premier agricultural units, and its budget

## Tapping a Deep, ‘Pre-Salt’ Bounty

Three years ago, a drill bit struck immense oil deposits deep off the coast of Brazil. Petrobras, the national oil company, tapped undersea fields now estimated to hold about 80 billion barrels of oil and natural gas—about three times the size of the reservoir under Prudhoe Bay, Alaska. It brought the promise of new wealth and expectations that Brazil will climb to the world’s top rung of achievement in science and technology.

Brazilian President Luiz Inácio Lula da Silva once termed the oil strike “a second independence for Brazil” and promised to use the oil revenue for education and public health. But Brazil’s R&D sector has been first to benefit. This

October, Petrobras inaugurated a sprawling new \$700 million research center in Rio de Janeiro. At the event, da Silva, a former union leader with a fourth-grade education, left no doubt what the vast R&D complex represents to him: “Brazil will never have to lower its head to anyone again,” he roared to a boisterous crowd of oil workers.

Deep-water petroleum exploration is Brazil’s largest technology project, and Petrobras’s money is pouring into research labs throughout the country. In order to retrieve the oil, which lies a daunting 7 kilometers below the ocean surface, Petrobras has opened a fire hose of funding that is “changing the face of science in Brazil,” says Angela Uller, dean for research at the Federal University of Rio de Janeiro, whose campus on an island outside the city also houses Petrobras’s R&D Center, known as Cenpes.

Petrobras now spends about \$1 billion a year on R&D, including some \$225 million that goes directly to universities, for which Petrobras has been rushing to outfit laboratories, erect new geophysics centers, and train a new generation of engineers. “We want to transform the technological capabilities of Brazil and help build university labs equal to any in the world,” says Carlos Tadeu da Costa Fraga, head of Petrobras R&D operations.

Rio’s engineering school, known as COPPE, is the biggest single beneficiary of the oil gusher. Petrobras has paid for the construction of numerous laboratories on campus, including the world’s deepest wave



**Oil money.** National oil company Petrobras inaugurated a \$700 million research center in Rio de Janeiro in October.



of \$1 billion is now the same size as that of the U.S. Department of Agriculture's Agricultural Research Service. "I've never seen so many resources for science as in the last 5 years," says Maria Fátima Grossi de Sá, a plant geneticist who recently received \$1.5 million to develop a transgenic cotton plant.

De Sá works at Embrapa's research station in Brasília, which is also finishing tests on a herbicide-resistant soybean that will be the first genetically modified crop designed by Brazilian scientists to reach the market. Demand for Ph.D. scientists is running so high that de Sá says it's difficult to find people to take postdoc positions. "We've gone pretty rapidly from having trouble placing Ph.D.s to having stipends that don't have takers."

Embrapa has nearly completed a four-story, \$15 million agro-energy center that will employ 100 researchers on the campus in Brasília. One goal is to turn Brazil's 22 million hectares of soybeans into more valuable products, such as biodiesel. "We capture solar energy and turn it into other forms of energy. We think we can move very quickly from agriculture for food to agriculture for energy. We can be a player," says Frederico Ozanan Machado Durães, general director of the new

unit. Countless shiploads of soybeans that embark for Asia every day from Brazilian ports could instead, he says, power domestic industries in lipochemistry and plastics that produce "value-added products."

The project represents an important shift in Brazilian thinking: namely, that science can transform the nation's economy, currently dominated by commodities like soy, beef, sugar cane, iron ore, and petroleum. "The new Brazil will be a natural knowledge economy," says Gilberto Câmara, head of Brazil's space agency.

With more money and an emerging green-science mission, Brazilian researchers say they're being taken more seriously. Most of Embrapa's senior scientists were trained in the United States, like Executive Director José Geraldo Eugênio de França, who in 1987 traveled to Texas A&M University to study sorghum genetics. De França says he noticed a change during a mission to Washington, D.C., last November, where he met U.S. science adviser John Holdren and other officials. "For the first time in history, we had a recognition that something was changing in Brazil. They didn't ask us how many postdocs we needed to send, or where

we needed help, but where could we work together," de França says.

### Private money

The most important goal right now, by Rezende's reckoning, "is for science to make a difference in the productivity of industry. I'd have to say that is our great challenge." Other goals are to increase the number of scientists, invest in strategic areas, and solve key social problems.

The disconnect between science and business is almost total in Brazil, researchers say. In the United States, about 80% of research personnel work in industry, according to OECD data, whereas in Brazil, that figure hovers near 25%. Brazil produces hardly any patents—just 103 U.S. patents were issued to inventors in Brazil in 2009—and Brazilian companies spend half of what European ones do on R&D. When they do spend, it's often to import technology rather than develop it.

Researchers say Brazil's 20-year dictatorship, which ended in 1984, is partly to blame for the lag. Universities became redoubts of political opposition and Marxist reading lists, where patents were viewed as oppressive. "We isolated ourselves from

pool, used to test automobile-sized models of oil platforms. "It's starting to look like Dubai around here," says Segen Farid Estefen, a director of COPPE, which gets about \$60 million a year from Petrobras. He says the industry-academic complex on the island is the "largest offshore oil research cluster in the world."

Petrobras, founded in 1947, began to follow the scent of oil offshore in the mid-1970s, investing in R&D to extend its reach. Brazil was importing equipment from the North Sea and the Gulf of Mexico and adapting it to tropical conditions. But Brazil's decision to pump its own oil demanded growing investment in R&D.

"You cannot simply cut and paste," says Martin Landrø, deputy chair of petroleum engineering and applied geophysics at the Norges Teknisk-Naturvitenskapelige Universitet in Trondheim, Norway. "You have to build up competence, and the easiest way to do that is to build up research. You have to bite the apple, so to speak."

Landrø, who has visited Brazil three times to give courses to Petrobras geophysicists, says he's noticed an accelerating change in Brazil. "They have maneuvered from the position of being not so competent to being on the cutting edge in 10 years," says Landrø.

Petrobras, the world's largest deep-water oil producer, is reaching depths where experience is scarce or nonexistent. At the Laboratory for Non-Destructive Testing, Corrosion and Soldering, for instance, four COPPE professors work alongside 30 Petrobras engineers to submit steel to corrosive hydrogen sulfide gas at extreme pressures. "At 7000 meters [below sea level], we don't have any information about how materials perform, or how long they can last," says Oscar Rosa Mattos, director of the lab, which Petrobras paid \$30 million to build in 2008. "My foreign visitors are surprised when they encounter a facility like this in Brazil."

The superdeep petroleum deposits now being discovered are in the "pre-salt" zone, an area where organic matter was deposited 125 million years ago



**Petro power.** With workers in 2008, President Luiz Inácio Lula da Silva celebrates the "second independence" oil discoveries will give Brazil.

and later encased beneath thick layers of salt. These are "a new kind of geologic play. They are new types of reservoirs and there are lots of things being learned," says William Fisher, a geologist at the University of Texas, Austin. One critical difficulty is spotting the oil reservoirs beneath the salt domes, frequently over a kilometer thick; seismic signals are hard to interpret. "As far as the potential discoveries—what is the potential volume of oil and gas—well, you can hazard all kinds of guesses, but it's going to be big," says Fisher.

Estefen hopes that Brazil's exploration of the ocean does not stop at oil. He says the country could use its deep-water expertise to be at the forefront of wave energy and undersea communications, too. "The analogy I use is that deep-sea exploration can do in Brazil what the space race did for the United States," Estefen says. "If Brazil only pumps oil, it would be a big loss." —A.R.



the big industries, which supported the military. They couldn't come in the university. The university became closed, hermetic, and now we have to change that," says Maria Bernardete Cordeiro da Sousa, dean for research at the Federal University of Rio Grande do Norte.

Officials have been trying to close the innovation gap. In 2004 and 2005, Brazil passed laws giving R&D tax breaks to companies and began allowing the Ministry of Science and Technology to give companies grants, even pay salaries of industrial researchers. In August, the ministry announced a major industrial R&D program, offering \$294 million in grants to back innovation projects inside companies in "strategic areas" including electric cars, pacemakers, and genetically engineered crops.

It's too early to say if the government incentives are working; only a small number of companies signed up for the tax breaks. But U.S.-style, risk-taking innovation, once viewed as alien, is increasingly seen in favorable terms. Venture capitalists have begun setting up shop in Brazil, and in 2010 both IBM and General Electric announced plans for research centers in the country.

"We lack a culture of innovation and entrepreneurship. There is a long path ahead to change that," says Luiz Mello, a physician who last year was tapped by Brazil's second largest company, the iron-ore miner Vale S.A., to spend \$180 million establishing three new corporate science institutes. Mello says he was hired after approaching Vale's CEO, Roger Agnelli, to raise money for an engineering program. "It turned out to be a meeting for him to say what he wanted. And



**Ph.D.-in-chief.** Physicist Sérgio Rezende has kept publishing while running Brazil's science ministry.

he wanted the MIT [Massachusetts Institute of Technology] of Vale," recalls Mello. "I was being invited to lead something that would be a new Bell Labs or Xerox PARC."

Mello traveled this fall to Silicon Valley to get ideas. Although Vale's business is low-tech, the commodity company, which ships vast amounts of ore to China and Europe, wants to spend heavily on research partly because it faces a sharp shortage of skilled labor, increasing pressure from environmentalists, and competition from global companies. Vale's three labs will work on biodiversity, renewable energy, and mining technology. "This is the biggest spontaneous investment in R&D that I know of in Brazil," says Mello.

The new laws also encourage Brazilian universities to file patents and set up technol-

ogy transfer offices, which many are doing for the first time. At the Federal University of Minas Gerais, the number of patent applications has reached 356, including one for a canine vaccine against leishmaniasis, now on the market. "All these things are leading to resonance in the system," says Ado Jorio, the professor who coordinates the university's patent efforts. "There has been an explosion in publications, and this is also going to happen in innovation."

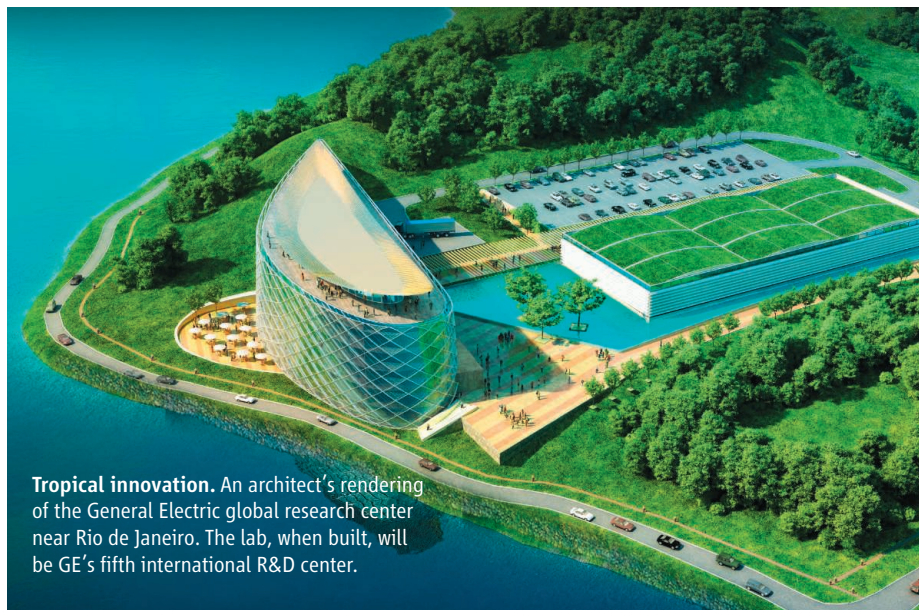
### Share the wealth

Brazilian science suffers another imbalance, between the wealthy south and the poor northern regions, that officials have put a priority on trying to correct. Most science still occurs in just three southern states, with the University of São Paulo alone accounting for nearly a quarter of all scientific publications. "One of the great questions we face is that Brazilian asymmetry, the inequality of the regions," says Lucia Melo, head of the Center for Strategic Studies and Management in Science, Technology and Innovation, a government science policy think tank in Brasília.

To push science out into Brazil's neglected hinterlands, Brazil's government has gone on a binge of university construction and earmarked 30% of research funds for poor northern and western states. Under a 2009 program called "Stipends for Everyone," officials in Brasília said they would give study grants to all graduate students in distant regions, regardless of academic merit.

The idea flows from Brazil's governing Workers' Party, which has made improving conditions in poor areas a priority. A greatly expanded welfare program has helped move several million Brazilians out of poverty. It has given researchers breathing room, too. "Before, we'd face the question, 'Why are you giving a monkey food and milk when there is a hungry child next door?'" says Cordeiro da Sousa, who is also a primate researcher. But she sees a tradeoff: Researchers feel increasing pressure to spend time solving local problems. She's considering creating a salt institute to support the local salt-mining industry. "You have to have a vocation, because in the future we could be called to answer in a big way."

Nowhere is the dearth of Brazilian-led science of greater concern than in the Amazon, the tropical forest that accounts for about 49% of Brazil's territory but is home to only about 3000 Ph.D. researchers, very few of whom do bench science. "Imagine what a totally irrelevant number that represents for this immense region," says Odenildo Teixeira Sena, secre-



**Tropical innovation.** An architect's rendering of the General Electric global research center near Rio de Janeiro. The lab, when built, will be GE's fifth international R&D center.

CREDITS (TOP TO BOTTOM): GE GLOBAL RESEARCH; WCT



tary of science and technology in the state of Amazonas. Although larger than France and Spain combined, Amazonas has only a single resident Ph.D. archaeologist, and despite its vast river systems, no naval engineers, Teixeira says.

Increasing scientific labor power in the region could help find alternatives to slash-and-burn agriculture. But national anxieties figure into the calculation as well. “The majority of publications on the Amazon don’t have a Brazilian author. That is a worry for us,” says Jorge Guimarães, the Ministry of Education official who oversees higher education in Brazil. “We need more Brazilians participating.”

Brazil has never felt secure in its control over the vast region, which Spain ceded to Portugal under the 1750 Treaty of Madrid. With the Amazon a focus of international maneuvering on carbon credits, Brazil’s dependence on foreign knowledge production has become a “very delicate question,” says Adalberto Val, director of the Instituto Nacional de Pesquisas da Amazônia in Manaus. During a national science and technology conference last May, Val called for Brazilian “informational hegemony” over the forest biome. “There is a question of national sovereignty,” he says.

Such nationalist tones may sound harsh outside Brazil, but they play well at home. Physicist Luiz Davidovich, who chaired the May conference, says Brazil’s scientific community needs to raise “big flags” to rally the country around. “‘The Amazon is Ours’ is one of those,” he says.

Even some foreign experts have responded to the call. Daniel Nepstad, a prominent American tropical forest ecologist, dropped his job in October at Woods Hole Research Center in Massachusetts to become a Brazilian resident and full-time employee of the Instituto de Pesquisa Ambiental da Amazônia, a nonprofit he co-founded, based in the city of Belém. Nepstad says his U.S. affiliation “was interpreted that I am less committed to the scientific agenda in Brazil.” Brazil’s forest policy is evolving rapidly and, Nepstad says, “as long as the science is led by Northerners, we are missing the opportunity to get really good information into policy decisions.”

### Making it real

Despite its growing ambitions, Brazil has yet to prove it can do world-class basic research. The impact scores of its scientific papers are modest, about two-thirds the world average, and have slid in some areas. No Brazilian has won a Nobel Prize in sci-



## Talented But Underfunded: Brazil’s Future Scientists

It’s an unusual prelab routine. Wake up in a below-ground apartment where five people sleep (four on the floor), grab your books, then head out past the machine gun–toting gangsters who guard your street against police vehicles. That’s been reality for 25-year-old Reinaldo Sousa dos Santos, a Ph.D. candidate in biochemistry at the Federal University of Rio de Janeiro (UFRJ) and a resident of Parque União, a crowded favela where residents live under the thumb of a drug gang.

Dos Santos owes his journey from shantytown to lab bench to his mentor, Leopoldo de Meis, a 72-year-old professor of biochemistry at UFRJ. In 1985, de Meis began offering a hands-on science course for low-income adolescents called Young Talents. Dos Santos enrolled when he was 14, a year after his father died, leaving him orphaned.

“I don’t want people to think I am the poor little kid from the favela,” says Dos Santos, a fast, concise talker who is studying the metabolic response of goldfish to low temperatures. Although he may sleep some nights on the laboratory couch, “intellectually I don’t think I am below anyone.”

Brazil must write thousands more stories like Dos Santos’s if it is to overcome deep social divisions and achieve its dream of becoming a major player in scientific research. Many say the task must begin with improving public schools, where poorly paid teachers offer rote lessons.

Brazil’s math and science scores vie for the worst among 57 countries ranked on the so-called PISA scale by OECD, barely edging out Tunisia. College is mainly for the elite. Prestigious federal universities in Brazil offer free education, but it’s difficult to pass tough entrance exams unless families have paid for private secondary schools. “We have had a perverse system of social apartheid, where the poor don’t have access to higher education,” says Luiz Davidovich, a physicist at the university who sits on the board of the Brazilian Academy of Sciences. Only 14% of 18- to 24-year-olds are in college, not enough to meet Brazil’s growing demand for researchers and engineers. “We’re working with just a sliver of the population,” Davidovich says.

In recent years, the government has tried to broaden access to education. Scores of state technical schools have opened; since 1979, Brazil has hosted what is now billed as the world’s largest math olympiad. Another national program, ProUni, since 2005 has paid for 748,000 lower income students who can’t get into federal schools to attend private, for-profit universities. Davidovich says the efforts fall short: “Brazil needs a revolution in education at all levels, especially at the most basic levels.”

Dos Santos recognizes that his life is exceptional. “Most Brazilians don’t know any scientists and don’t know what a scientist does,” he says. For him, science has proved both an intellectual and financial life belt. His state stipend of \$1000 a month (he gets another \$180 for supplies) makes him his family’s top earner, and this summer he helped his grandmother, uncle, and cousins move into a larger house. Now that he has his own room, he hopes to decorate it with a metabolic chart like one he saw at a conference.

“I like to see the molecule in vivo doing something,” says Dos Santos of his career choice. “The lab is really a psychological escape from all the situations I live through. It’s my ideal place.”

—A.R.





**Missed opportunity?** Many researchers have published studies involving the Amazon region; most are not Brazilian.

ence or medicine, whereas regional rival Argentina has three. Researchers blame structural problems at Brazil's state-run universities. Critics say they discourage competition, for example, with automatic tenure after 3 years on the job and evaluations that reward Portuguese-language publication. "The attitude for many years was to avoid competition, keep your head low, and choose a marginal subject," says Ribeiro. Instead of competing head-to-head on hot topics with big labs overseas, he says, Brazilian researchers have sometimes been content to study local questions. "The thinking was, 'The anteater is yours so don't worry about the gringos.'"

Brazilian researchers returning from overseas, drawn by jobs and start-up funds, complain that there are still many obstacles that make producing world-class science nearly impossible. After 11 years in the United States, biologist Luciana Relly Bertolini returned to Brazil in 2006 with her husband, Marcelo, to start a laboratory that aims to clone transgenic goats. Although the effort is adequately funded, Relly Bertolini says a heavy teaching load required of professors and lack of trained staff means "it's science by persistence here."

Also notorious are Brazil's Kafkaesque importation regulations. Even simple reagents can take months to arrive, with radioactive or biological samples often in doubtful condition. Relly Bertolini says a cell-fusion instrument she ordered from Hungary

**Returnee.** Tropical ecologist Dan Nepstad studies a controlled fire in Tanguro Forest, Mato Grosso, Brazil, in August.



has been trapped in customs for 4 months. "You can have the best head in the world and you will never be competitive because the government works against us," says Bertolini. "When we begin thinking that way, we want to go back."

Some say the prospects will remain bleak until such problems are solved. "I know of no extraordinary science in Brazil," says Andrew J. G. Simpson, scientific director of the Ludwig Institute for Cancer Research in New York City. A naturalized Brazilian citizen, he lived in São Paulo for 7 years and coordinated one of Brazil's memorable triumphs, the sequencing of the plant pathogen *Xylella fastidiosa*, which landed on the cover of *Nature* in 2000. But when Simpson returned this year for a 10th-year celebration of the feat, he noticed that, at least in the field of genomics, "there was never again a big impact paper. There was no upwards process. It was a blip."

Brazilian officials have instead focused on beating another problem: the insecurity of research funds. In 2008, in its largest-ever funding round for basic research, Brazil's Ministry of Science and Technology offered \$350 million over 3 years to fund 122 national institutes to tackle subjects from quantum computing and stem cells to an upgraded Antarctic research station. "They saw that we needed long-term programs with stability," says Davidovich, who co-leads the program on quantum computing.

Other scientists privately express doubts about the grandiosely named institutes, noting that in reality they are virtual networks with an average of 20 university investigators each and money spread too thin to achieve much. In position papers, the Brazilian Society for the Advancement of Science has said that Brazil needs to focus on creating more pure research jobs outside of the university system. It wants a new, heavily staffed state institute to study the oceans, and another for the Amazon, modeled on the agricultural agency Embrapa—in this case with funding to match the grandiose vision.

In the city of Natal, Nicolelis's neuroscience institute, currently housed in a converted hotel, has also yet to produce a Brazilian breakthrough. But it's increasingly well-positioned to do so. It has reasonably equipped laboratories, a primate facility, and a crowd of young professors with promising track records who have signed on, including two recruited from a Max Planck center in Germany. In August, the École Polytechnique Fédérale de Lausanne in Switzerland donated an IBM Blue Gene/L supercomputer, which Nicolelis says will be the fastest in South America.

Ribeiro, the Brazilian who returned from a postdoc at Rockefeller to be the institute's scientific director, says the year of science he expected to lose while organizing the center has dragged into three, as he faced down customs officials and coped with large numbers of poorly trained students. "Now I'm finally starting to fight reviewers again instead of bureaucrats, which is a sign the plan worked," says Ribeiro, whose work includes experiments to look at the effect of sleep and dreaming on motor and perceptual skill retention.

The dirt road outside his building that leads to a nearby shantytown, he says, reminds him of a photograph he saw of Rockefeller's Founder's Hall after it was built in 1906 and still surrounded by muddy fields and horse-drawn carriages: "They didn't start as the best place to do science either."

—ANTONIO REGALADO



## LETTERS

edited by Jennifer Sills

## Fishing for Data in the Ross Sea

WE ARE AMONG THE SCIENTISTS OBJECTING TO THE ECO-CERTIFICATION OF ROSS SEA ANTARCTIC toothfish (*Dissostichus mawsoni*), as described by E. Stokstad in his News Focus story "Behind the eco-label, a debate over Antarctic toothfish" (24 September, p. 1596). The public perceives a certification by the Marine Stewardship Council (MSC) to mean an environmentally friendly fishery, not one characterized by the "dearth of key data" as indicated in the article.

Significant data deficiencies lead us to conclude that an eco-friendly label for this fishery is scientifically indefensible. Credible life history data are missing: Spawning areas, eggs, and larvae have never been found, spawning intervals are unknown, and most density-dependent

aspects of ecological relationships are undetermined (1, 2). Stock assessment is problematic because severe Antarctic pack ice conditions for more than 9 months a year prevent scientists from effectively using standard models, which require random tagging over time, space, and age classes (3). The number of fish harvested by illegal, unregulated, and unreported fisheries is likely

substantial (4, 5). Finally, ecosystem effects of removing 50% of spawning biomass [the fishery's stated management goal (6, 7)] of this slow-to-mature species are unlikely to be neutral: The large, adult toothfish targeted by the fishery are a key structural link in the food web of the Ross Sea (8–11), currently the most pristine marine area on Earth (12).

As with MSC-certified fisheries elsewhere, toothfish certification requires that industry eventually provide missing biological data (13, 14). However, the harsh Antarctic environment makes data collection painstaking and often prohibitively expensive. Thus, such expectations are unrealistic for a commercially viable fishery. Instead of a certification that lacks proper data, a moratorium should be placed on further Ross Sea fishing until the quality of science at least equals that of certified fisheries elsewhere (13).

LOUISE K. BLIGHT,<sup>1</sup> DAVID G. AINLEY,<sup>2</sup> STEPHEN F. ACKLEY,<sup>3</sup> GRANT BALLARD,<sup>4</sup> TOSCA BALLERINI,<sup>5</sup> ROBERT L. BROWNELL JR.,<sup>6</sup> C.-H. CHRISTINA CHENG,<sup>7</sup> MARIACHIARA CHIANTORE,<sup>8</sup> DANIEL COSTA,<sup>9</sup> MALCOLM C. COULTER,<sup>10</sup> PAUL DAYTON,<sup>11</sup> ARTHUR L. DEVRIES,<sup>7</sup> ROBERT DUNBAR,<sup>12</sup> SYLVIA EARLE,<sup>13</sup> JOSEPH T. EASTMAN,<sup>14</sup> STEVEN D. EMSLIE,<sup>15</sup> CLIVE W. EVANS,<sup>16</sup> ROBERT A. GARROTT,<sup>17</sup> STACY KIM,<sup>18</sup> GERALD KOOYMAN,<sup>11</sup> AMÉLIE LESCOËL,<sup>19</sup> MICHAEL LIZOTTE,<sup>20</sup> MELANIE MASSARO,<sup>21</sup> SILVIA OLMASTRONI,<sup>22</sup> PAUL J. PONGANIS,<sup>11</sup> JOELLEN RUSSELL,<sup>23</sup> DONALD B. SINIFF,<sup>24</sup> WALKER O. SMITH JR.,<sup>25</sup> BRENT S. STEWART,<sup>26</sup> IAN STIRLING,<sup>27</sup> JAY WILLIS,<sup>28</sup> PETER WILSON,<sup>29</sup> ERIC J. WOEHLE<sup>30</sup>

<sup>1</sup>Centre for Applied Conservation Research, University of British Columbia, Vancouver, BC V6T 1Z4, Canada. <sup>2</sup>H. T. Harvey and Associates, Los Gatos, CA 95032, USA. <sup>3</sup>Geological Sciences Department, University of Texas San Antonio, San Antonio, TX 78249, USA. <sup>4</sup>PRBO Conservation Science, Petaluma, CA 94954, USA. <sup>5</sup>Center for Coastal Physical Oceanography, Old Dominion University, Norfolk, VA 23529, USA. <sup>6</sup>NOAA Fisheries, Southwest Fisheries Science Center, Pacific Grove, CA 93950, USA. <sup>7</sup>Department of Animal Biology, University of Illinois, Urbana, IL 61801, USA. <sup>8</sup>Università di Genova, Corso Europa, 26, 16132, Genoa, Italy. <sup>9</sup>Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, CA 95060, USA. <sup>10</sup>Specialist Group on Storks, Ibises, and Spoonbills, Chocorua, NH 03817, USA. <sup>11</sup>Scripps Institution of Oceanography, University of California, La Jolla, CA 92093, USA. <sup>12</sup>Department of Environmental Earth Systems Science, Stanford University, Stanford, CA 94305, USA. <sup>13</sup>National Geographic Society, Washington, DC 20090, USA. <sup>14</sup>Department of Biomedical Sciences, Ohio University, Athens, OH 45701, USA. <sup>15</sup>Department of Biology and Marine Biology, University of North Carolina, Wilmington, NC 28403, USA. <sup>16</sup>School of Biological Sciences, University of Auckland, PB 92019 Auckland, New

Zealand. <sup>17</sup>Ecology Department, Montana State University, Bozeman, MT 59717, USA. <sup>18</sup>Moss Landing Marine Laboratories, Moss Landing, CA 95062, USA. <sup>19</sup>Biodiversité et gestion des territoires, Université de Rennes 1, UMR 7204, Museum National d'Histoire Naturelle, 35042 Rennes Cedex, France. <sup>20</sup>Sustainability Office, University of Wisconsin, Oshkosh, 650 Witzel Avenue, Oshkosh, WI 54902, USA. <sup>21</sup>School of Biological Sciences and Gateway Antarctica, University of Canterbury, Christchurch, New Zealand. <sup>22</sup>Department of Environmental Sciences, Applied Ecology, University of Siena, 53100 Siena, Italy. <sup>23</sup>Department of Geosciences, University of Arizona, Tucson, AZ 85721, USA. <sup>24</sup>Department of Ecology, Evolution, and Behavioral Biology, University of Minnesota, St. Paul, MN 55108, USA. <sup>25</sup>Virginia Institute of Marine Sciences, College of William & Mary, Gloucester Point, VA 23062, USA. <sup>26</sup>Hubbs-SeaWorld Research Institute, San Diego, CA 92109, USA. <sup>27</sup>Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada. <sup>28</sup>HR Wallingford, Ltd., Oxfordshire OX10 8BA, UK. <sup>29</sup>17 Modena Crescent, Auckland, New Zealand. <sup>30</sup>School of Zoology, University of Tasmania, Sandy Bay, Tasmania 7005, Australia.

\*To whom correspondence should be addressed. E-mail: lkbright@interchange.ubc.ca

## References and Notes

1. A. L. DeVries, J. T. Eastman, "Brief review of the biology of *Dissostichus mawsoni*" (CCAMLR Doc WG-FSA-98/49, CCAMLR, Hobart, Australia, 1998).
2. S. M. Hanchet, G. J. Rickard, J. M. Fenaughty, A. Dunn, M. J. H. Williams, *CCAMLR Sci.* **15**, 35 (2008).
3. C. M. Brooks, J. R. Ashford, "Spatial distribution and age structure of the Antarctic toothfish (*Dissostichus mawsoni*) in the Ross Sea, Antarctica" (CCAMLR WG-FSA-08-18, CCAMLR, Hobart, Australia, 2008).
4. H. Österblom, U. R. Sumaila, Ö. Bodin, H. J. Sundberg, A. J. Press, *PLoS ONE* **5**, e12832 (2010).
5. TRAFFIC, "Australia confiscates 130 km long deepwater gillnet, Press Release 6, November" (Traffic International, Cambridge, 2009).
6. A. J. Constable, W. K. de la Mare, D. J. Agnew, I. Everson, D. Miller, *ICES J. Mar. Sci.* **57**, 778 (2000).
7. M. Pinkerton, S. Hanchet, J. Bradford-Grieve, *Water Atmos.* **15**, 20 (2007).
8. D. G. Ainley, G. Ballard, S. Olmastroni, *Aquatic Mam.* **35**, 335 (2009).
9. D. G. Ainley, D. B. Siniff, *Antarctic Sci.* **21**, 317 (2009).
10. J. T. Eastman, *Antarctic Fish Biology* (Academic Press, San Diego, CA, 1993).
11. W. O. Smith Jr., D. G. Ainley, R. Cattaneo-Vietti, *Philos. Trans. R. Soc. London Ser. B* **362**, 95 (2007).
12. B. S. Halpern et al., *Science* **319**, 948 (2008).
13. Marine Stewardship Council, "Net benefits' report" (September 2009); [www.msc.org/documents/fisheries-factsheets/net-benefits-report/](http://www.msc.org/documents/fisheries-factsheets/net-benefits-report/).
14. Marine Stewardship Council, "Ross Sea toothfish longline" (October 2010); [www.msc.org/track-a-fishery/in-assessment/southern-ocean/ross-sea-toothfish-longline](http://www.msc.org/track-a-fishery/in-assessment/southern-ocean/ross-sea-toothfish-longline).
15. The opinions of R.L.B. Jr. do not represent an official position or endorsement of third-party certification schemes for fisheries by NOAA and the U.S. Government.

## Assisted Colonization: Move Ahead with Models

MOVING SPECIES OUTSIDE THEIR NATURAL ranges has long been recognized as risky (“Home, home outside the range?”, R. Stone, *News Focus*, 24 September, p. 1592). Current accepted procedure allows for translocations outside the historic range only reactively—when there is no suitable habitat left in that range (1). For example, flightless birds such as kakapo and takahe have been introduced to offshore islands because exotic mammalian predators had rendered them unable to persist on the New Zealand mainland. Soon, we may have to move species proactively as a means to save them from anticipated shifts in habitat due to climate change. Proactive assisted colonization is understandably contentious. The best way forward involves careful modeling and collaboration.

There are many cautionary experiences from invasive species and biological control releases (2). However, tools such as structured decision-making enable us to make decisions in the face of uncertainty about ecological roles and relationships that we will have least cause to regret. We can construct models around the fate of species if we leave them to face climate change either by adapting, by moving, or by dying out. We can explore a deliberately moved species’ prospects of (i) dying out at its human-selected destination, (ii) establishing and becoming a pest, or (iii) settling down within desired population limits.

The test case in the News story of the two butterflies in the United Kingdom (3) is an example of the low-risk and potentially reversible type of experiment that we should be starting now. With assisted migration recognized as one means to reduce the impacts of climate change on biodiversity (4), we need international guidelines on the conditions under which it may be an acceptable solution. Consequently, the IUCN Species Survival Commission has established a task force from within its Re-introduction

and Invasive Species Specialist Groups to review and update its 1998 guidelines to explicitly accommodate these issues surrounding assisted colonization. The task force will report to the World Conservation Congress in 2012.

MARK R. STANLEY PRICE

Wildlife Conservation Research Unit, University of Oxford, Zoology Department, Oxford OX13 5QL, UK. E-mail: mark.stanleyprice@zoo.ox.ac.uk

### References

1. IUCN/SSC Re-introduction Specialist Group, “Guidelines for re-introductions” (IUCN, Gland, Switzerland, 1998).
2. A. Ricciardi, D. Simberloff, *Trends Ecol. Evol.* **24**, 248 (2009).
3. S. G. Willis *et al.*, *Conserv. Lett.* **2**, 45 (2009).
4. CBD, Conference of the Parties, “Biodiversity and climate change” (UNEP/CBD/COP/10/L.36, 2010).

## Assisted Colonization: Facilitate Migration First

IN HIS NEWS FOCUS STORY “HOME, HOME outside the range?” (24 September, p. 1592), R. Stone explores the risks and current data gaps associated with the practice of assisted colonization—actively translocating species from degrading ecosystems to locations more favorable to long-term survival. As Stone discussed, many scientists assert that the practice, also known as assisted migration, should play a substantial role in combating the effects of climate change on the survival of species that cannot successfully migrate or adapt. In addition to its risks and uncertainties, however, assisted colonization presents many ethical and legal issues (1), and may be more appropriate as the choice of last resort. An intermediate strategy between doing nothing and active translocation is facilitated migration, which involves securing the conditions necessary for successful species migration to and eventual settlement in more hospitable environments (2). Facilitated migration might include, for example, conserving migratory corridors and areas believed likely to transition into habitat suitable for a species’ new range.

Although it may not work for all climate-threatened species, such as those stranded on mountaintops by rising temperatures, facilitated migration presents none of the thorny ethical issues of assisted colonization and, if carried out properly, is perfectly legal under laws such as the Endangered Species Act. It also helps scientists focus on forward-looking planning and conservation to ensure that both the habitat of a species’ future and a way to get there are in place when needed. Facilitated migration should be developed in science and in policy as an important option for counteracting the effects of climate change on species

### Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web ([www.submit2science.org](http://www.submit2science.org)) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.



survival and, where possible, should be used before turning to assisted colonization.

J. B. RUHL

Florida State University College of Law, Tallahassee, FL 32306, USA. E-mail: jruhl@law.fsu.edu

#### References

1. A. E. Camacho, *Yale J. Reg.* **27**, 171 (2010).
2. J. B. Ruhl, *Natl. Wetlands Newsl.* **32**, 26 (July–August, 2010).

## Assisted Colonization: Protect Managed Forests

IN HIS NEWS FOCUS STORY “HOME, HOME outside the range?” (24 September, p. 1592), R. Stone presents a lucid view of the strengths and weaknesses of assisted colonization of endangered species. Unfortunately, the focus on assisted colonization is overshadowing far-reaching climate change adaptation programs targeting forests managed for producing timber, producing nontimber products, or stocking biomass to capture CO<sub>2</sub>.

In an effort to help managed forests respond to the effects of changes in climate, some propose the intentional translocation of tree species outside of their ranges. Forest managers seek to increase forest resilience by introducing new genotypes and new species (1). Social pressure to adjust managed forests in response to climate change should not be underestimated; managers are pushed to make decisions immediately, and

risks of introducing maladapted genes and invasive populations are inherent to this type of strategy. Some have even proposed introducing subtropical species from the southern hemisphere in northern temperate countries because of the species’ suitability to future warmer climates (2). As a result, exotic trees could be introduced legally into rural landscapes, thereby modifying terrestrial ecosystems for centuries in the name of responding to climate change.

We agree with Stone’s conclusion that scientists should closely advise programs considering assisted colonization, and we add a similar plea for managed forests programs. The attention paid to the ecological, ethical, and legal issues of assisted colonization of endangered species should not eclipse the risk assessment of natural and managed forest adaptation strategies.

JUAN F. FERNÁNDEZ-MANJARRÉS<sup>1,2,3\*</sup>

AND LOUISE TSCHANZ<sup>4</sup>

<sup>1</sup>Université Paris-Sud 11, UMR 8079, Orsay F-91405, France. <sup>2</sup>CNRS, Orsay F-91405, France. <sup>3</sup>AgroParisTech, Paris F-75231, France. <sup>4</sup>Faculté de Droit Jean Monnet, Université Paris-Sud, Bagneux F-92220, France.

\*To whom correspondence should be addressed. E-mail: juan.fernandez@u-psud.fr

#### References

1. E. Marris, *Nature* **459**, 906 (2009).
2. D. J. Read *et al.*, Eds., *Combating Climate Change—A Role for UK Forests: An Assessment of the Potential of the UK’s Trees and Woodlands to Mitigate and Adapt to Climate Change* (The Stationery Office, Edinburgh, 2009).

## CORRECTIONS AND CLARIFICATIONS

**News Focus:** “Killer bots are getting human” by J. Bohannon (1 October, p. 30). The game referred to as “Ultimate Tournament 2004” is actually named “Unreal Tournament 2004.”

**News of the Week:** “New type of cosmic dust tells of galaxy’s violent history” by Y. Bhattacharjee (24 September, p. 1590). The reference to “unprecedented images of the cloudshine feature” should have read “unprecedented images of the coreshine feature.”

**News Focus:** “Has China outgrown the one-child policy?” by M. Hvistendahl (17 September, p. 1458). In the graph “Having fewer babies anyway” (p. 1459), the top label on the y axis should have been 5, not 5%. The graph has been corrected in the HTML version online.

**Letters:** “Archaeology augments Tibet’s genetic history” by P. J. Brantingham *et al.* (17 September, p. 1467). The Letter referred to both the Report by T. S. Simonson *et al.* and the Report by X. Yi *et al.* Only the Report by X. Yi *et al.* should have been cited; T. S. Simonson *et al.* did not estimate a divergence time for high-altitude Tibetans.

**Policy Forum:** “Achieving scientific eminence within Asia” by A. S. Huang and C. Y. H. Tan (17 September, p. 1471). References 12 and 13 were incomplete. Reference 12 should be “Y. Shi, Y. Rao, *Science* **329**, 1128 (2010).” Reference 13 should be “S. Tole, R. D. Vale, *Science* **329**, 1441 (2010).” The references have been corrected in the HTML version online.

**Brevia:** “Island biogeography reveals the deep history of SIV” by M. Worobey *et al.* (17 September, p. 1487). A grant was omitted from the acknowledgment. The study was supported in part by Public Health Service grant RR000164.

**News Focus:** “The dour Frenchman on malaria’s frontier” by M. Enserink (3 September, p. 1142). The profile stated that combining an artemisinin-derived drug with another antimalarial was a novel concept in the early 1990s. In fact, others had explored that idea before. Researchers from the Guangzhou College of Traditional Chinese Medicine in Guangdong, China and the Roche Far East Research Foundation Hong Kong described the first clinical trial in which artemisinin was combined with other drugs: G. Q. Li, K. Arnold, X. B. Guo, H. X. Jian, L. C. Fu, *Lancet* **2**, 1360 (1984).

**News of the Week:** “NSF misfires on plan to revamp minority programs” by J. Mervis (23 July, p. 376). Stephen Cox was identified incorrectly. He is project director for the greater Philadelphia region Louis Stokes Alliance for Minority Participation program.

**Review:** “Development of monocytes, macrophages, and dendritic cells” by F. Geissmann *et al.* (5 February, p. 656). Reference 71 was incorrect. It should be A. Aziz *et al.*, *Science* **326**, 867 (2009).

## NEUROSCIENCE

# How Neuromythologies Support Sex Role Stereotypes

Diane F. Halpern

In a televised newscast this September, a medical correspondent for CBS News, Jennifer Ashton, M.D., explained that “men have six and a half times more gray matter than women do. Gray matter is partly responsible for information processing, so [this] may explain in general [why] men tend to be better in math” (1). Ashton went on to explain that women “have as much as 10 times as much white matter,” which could “contribute to why women are such good multi-taskers.” You don’t need to be a brain scientist to question these data, and you don’t need to know anything about sex differences in brain structures to recognize the long leap from neurons to math achievement or the ability to share attention among tasks. Carefully researched and reasoned, Rebecca Jordan-Young’s *Brain Storm* and Cordelia Fine’s *Delusions of Gender* offer antidotes to neuro-fallacies such as these.

The ease with which a popular media spokesperson can generalize from gray and white matter (which correspond to, respectively, cell bodies and myelinated axons) to why one sex might be better in math or in tasks that require shared attention is a perfect example of what Jordan-Young describes as an “infomercial” for cherished beliefs.” The physician-news reporter got just about everything wrong. Females and males have, on average, comparable ability in math (although there are more males than females in both the low and high ends of the ability distribution), and we can all get better at doing multiple tasks simultaneously with practice. Perhaps the most glaring flaw in her news report is what she left out: our brains change in response to experience, so any purported brain differences between males and females could have been caused by (and not the cause of) different life experiences. Even though this media sound bite fails on every dimension, it leaves viewers with the message that modern neuroscience can explain common stereotypes about the differences between females and males.

Similar in many ways, both books pro-

test what Fine (a psychologist at Macquarie University) calls “brain scams”: the irresponsible use of findings from the brain sciences to declare that females and males are essentially different and the assumption that we can use brain morphology as an explanation for sex-role stereotypes. Both authors rail against the claim that men are good at math and women are good at interpersonal tasks because they are hard-wired that way—a distinction that psychologist Simon Baron-Cohen has labeled synthesizing (male-typical) and empathizing (female-typical) brains (2). In addition to poking holes in the distinction between these two hypothesized brain types, Fine and Jordan-Young also enjoy critiquing extreme examples from the writings of psychiatrist Louann Brizendine, who promotes the idea that the differences in male and female brains can be seen in our everyday interactions (3). Both authors point out biases in the work of “essentializers” (people who believe that females and males are “essentially” and immutably different), and both emphasize how gender is learned, even at a young age. They take aim at researchers who seem blind to the cavernous

gap between some finding of sex differences in the brain and the relation of such distinctions to complex behaviors.

Although these books are more similar than different, each author has a particular slant to her message. Fine coined the term “neurosexism” to describe the use of neuroscience to support a sexist agenda that proclaims “girls are like this” and “boys are like that” because their brains work in different ways (4). It can aptly describe the work of some sloppy scientists and nonscientist popularizers who claim that they can explain on the basis of the neuroanatomy of male and female human brains why men don’t ask for directions or why women gossip.

The erudite term, however, does not apply to everyone who studies sex differences in the brain. Many of the scientists who study neuroanatomical sex differences are responsible in the way they conduct research and cautious about the conclusions they make.

Despite the large amount of junk science on the topic that is reported in the popular media and in some academic outlets, there are also consistent findings of sex differences that hold up across studies, across species, and across cultures. Most of these are ignored by Fine. This may, in part, reflect the fact that whereas sex differences are reliably found in several areas of research, none of the differences support essentialist claims that girls and boys need separate educations based on their brain types, that one sex is better suited to become engineers, or that one sex is inherently more intelligent—to name just a few of the ideas being promoted under the guise of “science.” For example, in a recent set of

studies that collected data from over 200,000 men and women via a BBC Web site, a test of estimating the orientation of lines and other visuospatial tasks found medium to large sex differences favoring males across 53 countries (5). On the other hand, training studies have found that everyone can improve on visuospatial tasks (6), so any explanation must consider the various ways in which biological and psychosocial factors affect one another.

Consider a biopsychosocial model in which individuals are predisposed by their biology to learn certain skills more readily than others while everyone selects experiences that are biased by prior learning histories,

## Brain Storm

The Flaws in the Science of Sex Differences

by Rebecca M. Jordan-Young

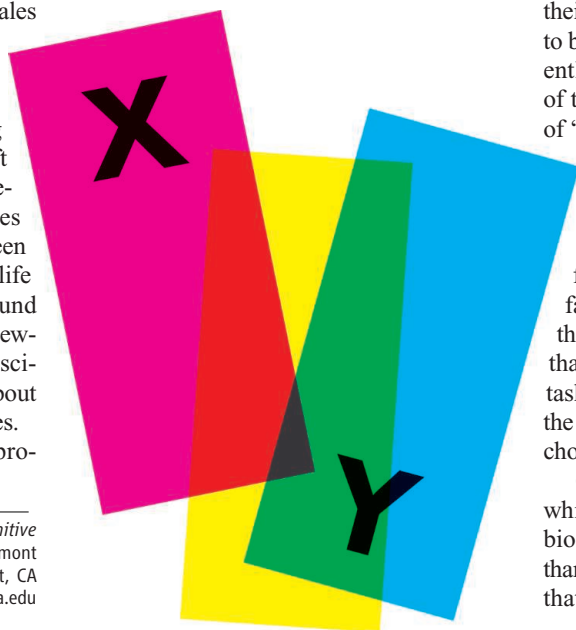
Harvard University Press, Cambridge, MA, 2010. 408 pp. \$35, £25.95, €31.50. ISBN 9780674057302.

## Delusions of Gender

How Our Minds, Society, and Neurosexism Create Difference

by Cordelia Fine

Norton, New York, 2010. 368 pp. \$25.95, C\$32.50. ISBN 9780393068382.



The reviewer, the author of *Sex Differences in Cognitive Abilities*, is at the Department of Psychology, Claremont McKenna College, 850 Columbia Avenue, Claremont, CA 91711, USA. E-mail: diane.halpern@claremontmckenna.edu



opportunities afforded in their environments, and beliefs about appropriate behaviors for females and males. Experiences change neural structures, which in turn alter how individuals respond, and so on. As many stereotypes about sex differences reflect group differences between males and females, by learning and endorsing them, individuals may also be selecting environments and experiences that increase or reduce these differences.

A relatively recent paradigm shows just how complicated sex differences can be. Several different researchers examined the way sex differences vary as a function of the gender equality across societies. Consider the finding that, in more gender-equal societies, females perform as well as males in mathematics (7), much better than males in reading (7), and much worse than males in visuospatial tasks (5). No simple theory, such as the hypothesis that sex differences reflect societal norms or that gender-equal societies will reduce all sex differences, can explain this pattern of results.

Not all claims of biological influences are wrong or wrong-headed. On the simplest level, there is a genetic basis for some types of mental retardation that are more common among males than among females. There are numerous gonadal hormone receptors in the brain, and such hormones do influence some sex-typed behaviors, both prenatally and, to a lesser extent, after puberty. Both books lack a more nuanced approach that might teach readers how to distinguish solid research on hormonal and genetic influences of male and female behavior from overhyped and simple-minded claims. It is much more difficult to delve into the sometimes messy details that good research must address than it is to take a one-sided approach that highlights only the bad work. Unfortunately, it is also less entertaining to read about a balanced approach to complex questions. Nonetheless, the more balanced view more accurately reflects the current state of research on sex differences.

The subtitle of *Brain Storm* characterizes its focus on “the flaws in the science of sex differences.” There are many, but like Fine, Jordan-Young (a sociomedical scientist at Barnard College) sometimes fails to distinguish between valid research claims and psychobabble. Throughout the book, the author explores three main themes: sex, gender, and sexuality. She wisely reminds us that context is a critical variable in understanding these three strands of human experience, and like Fine, she emphasizes socialization.

She takes particular aim at research findings from girls afflicted with congenital adrenal hyperplasia, a family of autosomal

recessive disorders that impair the synthesis of cortisol in the adrenal glands. Most cases involve an enzyme deficiency that causes the adrenal glands to excrete excessive levels of masculinizing hormones throughout gestation. Jordan-Young notes the contradictory findings from this atypical population, and she suggests that the unique experiences of these girls could underlie their unusual gender responses. She also assails the notorious case of a child who was raised as a girl after an accident destroyed his penis during routine circumcision when he was seven months old. Her criticisms are well-founded, but she also ignores much relevant high-quality research by scientists who take care in their work and in the scope of their claims. For some examples: Jay Giedd (National Institute of Mental Health) has found sex differences in normal brains at every stage of development (8). Larry Cahill (University of California, Irvine) has shown that sex and brain lateralization are important influences on emotion and memory (9). Bruce McEwen (Rockefeller University) has spent decades carefully documenting estrogen and other hormonal effects on neural development (10).

Cleverly written with engaging prose, *Delusions of Gender* and *Brain Storm* contain enough citations and end notes to signal that they are also serious academic books. Fine and Jordan-Young ferret out exaggerated, unreplicated claims and other silliness regarding research on sex differences. The books are strongest in exposing research conclusions that are closer to fiction than science. They are weakest in failing to also point out differences that are supported by a body of carefully conducted and well-replicated research. The question is not whether female and male brains are similar or different, because they are both. The questions we need to answer are: How can we understand the ways in which we are similar and different? And how can we use that knowledge to help everyone achieve their fullest potential?

#### References

1. [www.cbsnews.com/stories/2010/09/22/earlyshow/health/main6890474.shtml?tag=cbsnewsSectionContent.9; updated 22 September 2010](http://www.cbsnews.com/stories/2010/09/22/earlyshow/health/main6890474.shtml?tag=cbsnewsSectionContent.9; updated 22 September 2010).
2. S. Baron-Cohen, *The Essential Difference: Men, Women, and the Extreme Male Brain* (Allen Lane, London, 2003).
3. L. Brizendine, *The Female Brain* (Bantam, London, 2007).
4. C. Fine, *Neuroethics* **1**, 69 (2008).
5. R. A. Lippa et al., *Behav.* **39**, 997 (2010).
6. D. Tzuriel, G. Egozi, *Child Dev.* **81**, 1417 (2010).
7. L. Guiso et al., *Science* **320**, 1164 (2008).
8. [http://intramural.nimh.nih.gov/research/pi/pi\\_giedd\\_j.html](http://intramural.nimh.nih.gov/research/pi/pi_giedd_j.html).
9. <http://cahill.bio.uci.edu/>.
10. <http://rockefeller.edu/research/faculty/abstract.php?id=109>.

10.1126/science.1198057

## THEATER: HISTORY OF SCIENCE

# Shades of Gray in DNA Drama

Barbara Juncosa

**K**ing's College, London, 1952: Rosalind Franklin develops x-ray diffraction photograph number 51. Illuminated by her light box, a crisp striped "X" stares back at her from the center of the photo. In this brief moment, Franklin reveals the helical nature of DNA and cements a legacy doomed to controversy.

Anna Ziegler's play *Photograph 51* dramatizes the race to determine the structure of DNA—the Nobel Prize-winning work that produced one of the most important scientific discoveries of the 20th century. The narrative centers on Rosalind Franklin, who is presented as a prickly biophysicist obsessed with capturing the perfect image needed to resolve the molecule's structure.

The play opens as Franklin arrives at King's College, where she faces anti-Semitic and sexist sentiments from fellow researchers. But Franklin's combative temperament and secrecy soon become the focus of the story. Although Franklin ultimately succeeds in capturing the elusive structure on film, she refuses to share her work with colleague Maurice Wilkins or even construct a model before completing her detailed calculations.

Alienating Wilkins with her repellent disposition, Franklin inadvertently drives him to secretly disclose photograph 51 to James Watson. Realizing the significance of Franklin's image, Watson returns to the laboratory of Francis Crick, where the two complete the model of double-stranded DNA presented in their 1953 *Nature* paper. Franklin's contribution, however, goes largely unacknowledged by her male colleagues.

Despite the harsh portrayal by playwright Anna Ziegler (1), Franklin's unwavering dedication to the pursuit of truth makes her an admirable character. Rather than dwell on her defeat, Franklin celebrates humanity's victory over the mysteries of life. Even as she fights a losing battle with ovarian cancer at the age of



Kristen Bush in the role of Rosalind Franklin.

38, her research remains the priority. The play culminates in a stirring confrontation with Wilkins in which he—confessing his love for Franklin—laments the multitude of missed opportunities between the two scientists.

*Photograph 51* provides an emotional journey into the complex realities of laboratory science. Ziegler portrays scientists who—far from the rational, white-coated automatons of popular culture—struggle with personal ambitions, intense competition, and rocky relationships as they strive to define the material of heredity. The small stage provides a dynamic environment for the characters, who often observe Franklin's progress from the sidelines, arguing their viewpoints directly to the audience. The play's director, Lindsay Firman, makes use of the cramped space to create striking juxtapositions between the theorists Watson and Crick fiddling with their models and the staunch experimentalist Franklin scribbling calculations in her notebook.

The play raises a number of important issues, from the role of women in science to the importance of scientific collaboration. To discuss these themes, the Ensemble Studio Theatre hosted a lively panel discussion 2 November at the Julia Miles Theater in New York (2). Although James Watson could not attend as planned, tense moments still emerged following the assertion by Nicholas Wade, the *New York Times* science reporter, that "the idea that [Franklin] was robbed of credit is incorrect." Biologist

and Franklin scholar Lynne Osman Elkin (3) shook her head in disagreement, vehemently arguing that Franklin would "not hand unpublished data to a competitor. Period."

The panel moderator, neuroscientist Stuart Firestein, posited that the historical narrative might have been affected by glaring differences in the tone and quality of the two groups' scientific communiqués. "The Watson and Crick paper is, I have to say, a work of scientific art," he commented. "You see immediately what they saw ... It lets you into the beauty of the whole molecule. And Franklin's paper is extremely informative, but if you're not

a crystallographer, you're not going to get anywhere through this paper."

Although the controversy over Franklin's rightful credit will continue to be debated, the panelists agreed that photograph 51 provided key data for Watson and Crick's DNA model. Despite the inequalities between male and female scientists at King's College, the panelists contended that the men involved in the DNA race did not discriminate against her because she was a woman or Jewish. Rather, they suggested that her severe disposition and impossible relationship with Wilkins likely stymied her progress.

In the end, *Photograph 51* provides a compelling perspective on Franklin in which even the complex science is engaging and accessible. Whether she was a wronged heroine or quarrelsome perfectionist, her contributions to the human understanding of life's hereditary material are difficult to understate. She passed away before the Nobel Prize was awarded to Crick, Watson, and Wilkins in 1962. But even had she survived until then, one wonders whether the scientific community would have recognized her substantial role in the revolutionary breakthrough.

## References and Notes

1. Those seeking a nonfictional portrait of Franklin should turn to Brenda Maddox's nuanced biography (4).
2. A two-part podcast of the panel discussion is available at [www.scientificamerican.com/podcast/episode.cfm?id=photograph-51-rosalind-franklin-and-10-11-03](http://www.scientificamerican.com/podcast/episode.cfm?id=photograph-51-rosalind-franklin-and-10-11-03) and [www.scientificamerican.com/podcast/episode.cfm?id=photograph-51-rosalind-franklin-and-10-11-05](http://www.scientificamerican.com/podcast/episode.cfm?id=photograph-51-rosalind-franklin-and-10-11-05).
3. L. O. Elkin, *Phys. Today* **56**, 42 (2003).
4. B. Maddox, *Rosalind Franklin: The Dark Lady of DNA* (HarperCollins, London, 2002); reviewed in (5).
5. A. Fausto-Sterling, *Science* **298**, 1177 (2002).

The reviewer is at the Laboratory of Bacterial Pathogenesis and Immunology, Rockefeller University, 1230 York Avenue, New York, NY 10065, USA. E-mail: [bjuncosa@gmail.com](mailto:bjuncosa@gmail.com)

10.1126/science.1199778



# Developing Health Workforce Capacity in Africa

Francis S. Collins,<sup>1</sup> Roger I. Glass,<sup>2\*</sup> Jack Whitescarver,<sup>3</sup> Mary Wakefield,<sup>4</sup> Eric P. Goosby<sup>5</sup>

As we mark World AIDS Day, it is important to assess the relationship between the challenges of AIDS prevention and control and the huge gaps in the health workforce needed to address these and other critical shortages. Although Africa bears 24% of the global disease burden, it has only 3% of the world's health workforce and less than 1% of the world's financial resources for health (1, 2). Sub-Saharan Africa as a whole has 18 physicians per 100,000 population, ranging from 60 in South Africa to 2 in Mozambique. Meanwhile, Africa struggles to retain its precious health workers. Approximately 65,000 African-born physicians, or about one-fifth of those trained, have migrated to high-income countries within 5 years of completing their training (3). Moreover, one-third of medical school faculty posts are vacant, and 30% of faculty must supplement their income to continue teaching (4). It is vital to increase the quality and quantity of local health care workers, particularly in remote rural areas. Scientific expertise must be enhanced to determine the best prevention and treatment strategies for the local context, as well as improve target countries' (5) ability to monitor and evaluate outcomes.

The U.S. President's Emergency Plan for AIDS Relief (PEPFAR) has recently partnered with the National Institutes of Health (NIH) and the Health Resources and Services Administration (HRSA) in an initiative to transform medical education at select medical schools in Africa (6). The Medical Education Partnership Initiative (MEPI) includes collaboration with the U.S. Agency for International Development, the Centers for Disease Control and Prevention, and the Department of Defense and is intended to provide a focus for U.S. agencies to support local health ministries and academic centers to improve the quantity, quality, and retention of their graduates and improve their research capabilities.

The MEPI awards were announced in October 2010 and will invest about \$130 million over 5 years to directly support African institutions in 12 countries, creating a network of 30 regional partners and more than 20 U.S. collaborating institutions (7) (see the table). For example, in South Africa, the two proposals are directed at two of the hardest hit areas: Stellenbosch University is working on the training of physicians to work in rural areas with the least resources by recruiting students from these underserved areas. The University of KwaZulu-Natal is located in a region with the highest rates of HIV and tuberculosis in South Africa and has been a training ground and research center for the region. Researchers there are conducting large-scale interventions (e.g., circumcision) and have had a history of supporting research training for physicians from surrounding countries and former homelands, where non-whites were segregated during apartheid.

The MEPI program was developed after consultation with African leaders and international donors, and the sites were selected based on competitive peer review by NIH. The Office of the Global AIDS Coordinator (OGAC) will provide about \$105 million for AIDS-related training; the Office of AIDS Research at NIH will provide an additional \$10 million; and the NIH Director's Common Fund will provide \$15 million to extend training into other critical areas such as maternal, neonatal, and child health and noncommunicable diseases. By engaging national ministries of health and education, participating institutions will leverage MEPI to strengthen national plans to improve medical education, expand the quality and scope of instruction, and scale up the existing cadre of health care workers. In addition to increasing the quality and quantity of health care workers, MEPI will develop the in-country scientific expertise needed to conduct research on implementing programs and identify novel solutions to the most pressing problems to bridge gaps in the delivery of care. Of the current grants, 80% plan to incorporate community-based education and/or rural training, 73% target nurses and nursing students for enhanced training (schools of pharmacy and dentistry are included in some of the pro-

In Africa, programs to improve health will require major investments in institutions that can train and retain health professionals.

posals), 64% plan to have offerings in public health (to include biostatistics, epidemiology, and the like), 45% propose strategies to increase the number of women clinicians and researchers, and 36% of programmatic awards are intended to include maternal-child health training and research in the context of HIV/AIDS (including training of midwives).

This venture is aligned with the principles of President Obama's Global Health Initiative (8). MEPI is among several programs to support the congressional mandate that PEPFAR help partner countries train and support the retention of at least 140,000 new health care workers at all levels to strengthen the capacity in developing countries, particularly in sub-Saharan Africa (9). Finally, this will allow an explicit intervention priority—HIV/AIDS—to drive overall improvements in the local health systems and provide care for a broader range of conditions, as recommended by the Institute of Medicine (10).

PEPFAR's initial emergency approach—while necessary early on—has had both positive and negative impacts on country-level health systems and budgets. In moving forward to quickly establish a service capacity, implementation did not always take into account existing national health care structures or plans. Nor did the focus on HIV/AIDS carefully translate to broader service and delivery needs such as training and providing health workers to peripheral areas or family planning and child health. This partnership, with its emphasis on long-term response represents a new direction for PEPFAR (11).

Many participants will devote funds to strengthen mentoring, peer-to-peer training, and hands-on clinical research experience. Through MEPI, medical schools in sub-Saharan Africa will be able to develop and expand novel models of education, recruit and retain qualified faculty, and increase the capacity for locally driven, multidisciplinary research. They will also be able to broaden their curricula to develop and expand training focused on maternal and child health, emergency medicine, cardiovascular disease, mental health, and HIV-related cancers. Because Africans with HIV/AIDS are now living longer, thanks to antiretroviral drugs, they are developing chronic diseases in growing numbers, yet

<sup>1</sup>National Institutes of Health (NIH), Bethesda, MD 20892, USA. <sup>2</sup>Fogarty International Center, NIH, Bethesda, MD 20892, USA. <sup>3</sup>Office of AIDS Research, NIH, Bethesda, MD 20892, USA. <sup>4</sup>Health Resources and Services Administration, Rockville, MD 20857, USA. <sup>5</sup>Office of Global AIDS Coordinator, Washington, DC 20522, USA.

\*Author for correspondence. E-mail: glassr@mail.nih.gov

## MEPI PARTNERS

| Country and Institutions                                                                                                                                                                                   | U.S. Universities and Other Partners                                                                                  |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------|
| <b>Botswana</b><br>University of Botswana*                                                                                                                                                                 | Harvard University<br>University of Pennsylvania                                                                      |
| <b>Ethiopia</b><br>Addis Ababa University*<br>Hawassa and Haremaya Universities<br>Defense Forces Medical Colleges                                                                                         | Emory University<br>Johns Hopkins University<br>University of Wisconsin<br>University of California, San Diego (UCSD) |
| <b>Kenya</b><br>University of Nairobi*                                                                                                                                                                     | University of Maryland, Baltimore<br>University of Washington, Seattle                                                |
| <b>Mozambique</b><br>University of Eduardo Mondlane*<br>Universities of Lurio and Zambeze                                                                                                                  | UCSD and World Health Organization<br>Canadian Network for International Surgery<br>American College of Surgeons      |
| <b>Nigeria</b><br>University of Ibadan*<br>Universities of Jos, Nigeria, Maiduguri, Ahmadu Bello, and Lagos<br>AIDS Prevention Initiative Nigeria, Ltd.                                                    | Northwestern University<br>Harvard University                                                                         |
| <b>South Africa</b><br>University of KwaZulu-Natal*                                                                                                                                                        | Columbia University                                                                                                   |
| <b>South Africa</b><br>Stellenbosch University*<br>University of Cape Town<br>Makerere University (Uganda)                                                                                                 | Johns Hopkins University                                                                                              |
| <b>Tanzania</b><br>Kilimanjaro Christian Medical Centre*                                                                                                                                                   | Duke University                                                                                                       |
| <b>Uganda</b><br>Makerere University*<br>Mbarara, Kampala International, Busitema, and Gulu Universities<br>Medical Research Council                                                                       | Johns Hopkins University<br>Case Western Reserve University<br>Yale University                                        |
| <b>Zambia</b><br>University of Zambia*                                                                                                                                                                     | Vanderbilt University<br>University of Alabama, Birmingham                                                            |
| <b>Zimbabwe</b><br>University of Zimbabwe*<br>University of Cape Town (South Africa)                                                                                                                       | University of Colorado, Denver<br>Stanford University<br>University College and King's College, London                |
| <b>Ghana</b><br>Kwame Nkrumah University of Science and Technology*<br>Ghana Ministry of Health and Komfo Anokye Teaching Hospital<br>Ghana College of Physicians and Surgeons and Ghana Ambulance Service | University of Michigan                                                                                                |
| <b>Malawi</b><br>University of Malawi College of Medicine*<br>University of Cape Town (South Africa)                                                                                                       | University of North Carolina<br>Johns Hopkins University                                                              |
| <b>Coordinating Center</b><br>The African Center for Global Health and Social Transformation (Uganda)                                                                                                      | George Washington University*                                                                                         |

\*Principal grantee.

there are very few African health care workers trained in these specialties. Deaths from chronic diseases are rising quickly in Africa. The World Health Organization is forecasting a 27% increase over the next decade (12).

MEPI will also support establishment of a network (via a Web-based platform) to link African sites and U.S. collaborators, leverage resources, and provide technical expertise. Initially, the network will be coordinated by the George Washington University School of Public Health, in partnership with HRSA, NIH, and OGAC, and will monitor progress and identify best practices. In 5 years, many of these functions will be transferred to the Center for Global Health and Social Transformation in Kampala, Uganda. Our goal is to empower the sites to pursue their own priorities and network within the program and their own countries.

This initiative will enable participating institutions to strengthen their information technology infrastructure to enhance educational and research activities, including increasing bandwidth and computer access, introducing intranets and other shared resources, and encouraging mobile technology platforms for information delivery and data collection for clinical research. MEPI will also support distance education and data sharing that will encourage much-needed clinical registries to inform health care decisions and research needs on national levels.

This initiative is not without challenges. Given current faculty shortages and infrastructural deficits at many schools, assuring quantity and quality scale-up and retention strategies will require disciplined efforts on the part of all involved. Success will necessitate coordination with ministries of health

and education on a country by country basis.

The ultimate goal is to create long-term capacity at Africa's educational institutions to produce the quantity and quality of health care workers and scientists required and broaden the training provided to cover not only HIV-related disease but also issues of maternal and child health, noncommunicable diseases, and other national priorities. Measurable outcomes should be a substantial increase in the numbers and quality of physicians and health professionals trained and retained in country (many working in underserved areas), more robust residency training programs, and removal of silos between AIDS and non-AIDS care. The only means to bring our efforts to effective scale is through creative partnerships with other governments, philanthropies, international organizations, and industry. With the launch of MEPI, we welcome cooperation to build synergies and accelerate our shared objectives and we will be reaching out to prospective partners. A generation of skilled African professionals trained to apply evidence-based solutions for improved health would be a lasting legacy.

## References

1. World Health Organization, Report on the WHO/PEPFAR Planning Meeting on Scaling Up Nursing and Medical Education, Geneva, 13 to 14 October 2009; [www.who.int/hrh/resources/scaling-up/en/index.html](http://www.who.int/hrh/resources/scaling-up/en/index.html).
2. Global Health Workforce Alliance, *Scaling Up: Saving Lives* (World Health Organization, Geneva, 2008); [www.who.int/workforcealliance/documents/Global\\_Health%20FINAL%20REPORT.pdf](http://www.who.int/workforcealliance/documents/Global_Health%20FINAL%20REPORT.pdf).
3. International Organization for Migration, *World Migration 2005* (International Organization for Migration, Geneva, 2005).
4. F. Mullan et al., *The Sub-Saharan African Medical School Study: Data, Observation, and Opportunity* (SAMSS, Washington, DC, 2010).
5. [www.pepfar.gov](http://www.pepfar.gov).
6. National Institutes of Health, News release: HHS Partners With PEPFAR to Transform African Medical Education with \$130M Investment, October 2010; [www.fic.nih.gov/news/publications/global\\_health\\_matters/2010/1010\\_medical-education-africa.htm](http://www.fic.nih.gov/news/publications/global_health_matters/2010/1010_medical-education-africa.htm).
7. National Institutes of Health, Directory of Awards and Collaborating Partners for the Medical Education Partnership Initiative (MEPI), October 2010; [www.fic.nih.gov/programs/training\\_grants/mepi/awards.htm](http://www.fic.nih.gov/programs/training_grants/mepi/awards.htm).
8. Implementation of the Global Health Initiative: Consultation Document (1 February 2010); [www.usaid.gov/our\\_work/global\\_health/home/Publications/docs/ghi\\_consultation\\_document.pdf](http://www.usaid.gov/our_work/global_health/home/Publications/docs/ghi_consultation_document.pdf).
9. Tom Lantos and Henry J. Hyde United States Global Leadership Against HIV/AIDS, Tuberculosis, and Malaria Reauthorization Act of 2008, Pub. L. No. 110-293 (2008).
10. Institute of Medicine, *PEPFAR Implementation: Progress and Promise* (National Academies Press, Washington, DC, 2007); [www.nap.edu/catalog.php?record\\_id=11905](http://www.nap.edu/catalog.php?record_id=11905).
11. The U.S. President's Emergency Plan for AIDS Relief: Five-Year Strategy (2009); [www.pepfar.gov/strategy/document/index.htm](http://www.pepfar.gov/strategy/document/index.htm).
12. World Health Organization, 2008–2013 Action Plan for the Global Strategy for the Prevention and Control of Noncommunicable Diseases (WHO, Geneva, 2008); [www.who.int/nmh/Actionplan-PC-NCD-2008.pdf](http://www.who.int/nmh/Actionplan-PC-NCD-2008.pdf).

10.1126/science.1199930



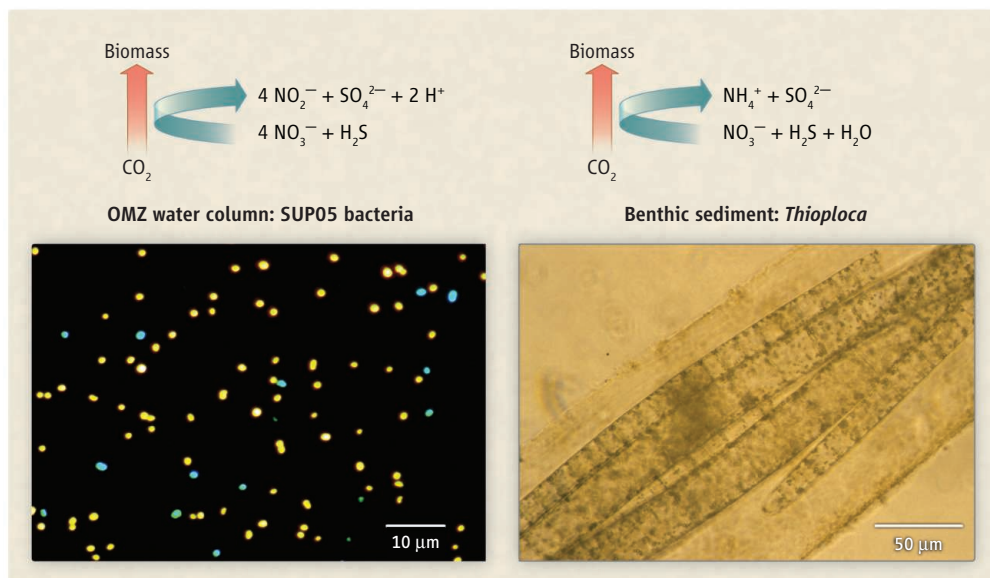
## OCEANS

## Cryptic Links in the Ocean

Andreas Teske

Oxygen depletion in the ocean water column, in stratified basins such as the Santa Barbara and Cariaco basins, and in enclosed anoxic oceans such as the Black Sea provide vivid examples of a type of marine habitat where multicellular life gradually disappears with depth as oxygen is consumed by respiration and not replenished. Only bacteria and archaea with anaerobic metabolisms persist, controlling the microbial cycling of nitrogen, sulfur, metals, and carbon. Genuine fondness for these environments and their anaerobic microbial inhabitants, although possibly an acquired taste, is widespread among marine microbiologists and geochemists who are tracing the path of key elements and the role of microbes in the ocean's biogeochemical economy. On page 1375 of this issue, Canfield *et al.* (1) analyze the microbial cycling of nitrogen and sulfur in the oxygen minimum zone offshore of northern Chile. They show that our working hypotheses on microbial interactions and processes of stratified marine water columns have overlooked a critical component: the contribution of the "cryptic" microbial sulfur cycle.

Why cryptic? In principle, microbial processes should reveal themselves by the reaction partners that are produced and consumed. For example, the dominant anaerobic respiration pathway in the marine environment, sulfate reduction—the use of the seawater anion sulfate for respiration by specialized groups of bacteria and archaea—generates the end product sulfide. The gradual disappearance of sulfate, concomitant with sulfide production, would be a reliable indicator for microbial sulfate reduction. Yet studies of marine sediments have already shown that these microbial processes are easily camouflaged; sulfide accumulation is often held in check by specialized sulfide-oxidizing bacteria, as long as suitable oxidants are available. For example, the continental shelf of Chile and Peru harbors extensive microbial mats of



**Ocean biochemistry.** Simplified scheme of sulfur and nitrogen cycle linkages for pelagic SUP05 bacteria, contrasted with benthic filaments of *Thioploca*. Lower left, epifluorescence in situ hybridization image of SUP05 cells, appearing in yellow, from the Suiyo Seamount hydrothermal plume; lower right, bright-field image of sheathed benthic *Thioploca* filaments from shelf sediments underlying oxygen-depleted water near Concepción, Chile.

filamentous bacteria, the sulfide-oxidizing, nitrate-reducing *Thioploca* species (2, 3). In conjunction with a highly active microbial iron cycle in the upper sediments, the *Thioploca* mats reoxidize sulfide so efficiently that the amount of sulfide actually remains near the detection limit within the mat, despite an extremely high sulfate reduction rate in the immediately surrounding sediment (4). The water column of northern Chile contains an oxygen minimum zone (OMZ) in which sulfate reduction accounts for a surprisingly large proportion of total carbon remineralization. However, the resulting sulfide is reoxidized so efficiently that it does not accumulate, effectively camouflaging sulfate-reducing activity in the water column. In both habitats, sediment and water column, cryptic sulfur cycling does not mean that the process is environmentally irrelevant. On the contrary, preventing the buildup of free sulfide by instant reoxidation is essential to counter sulfide toxicity.

Canfield *et al.* link cryptic, nitrate-driven sulfur cycling to characteristic sulfur-oxidizing microbial communities of the Chilean OMZ. Previously, this bacterial community had been identified by 16S rRNA gene sequencing (5); Canfield *et al.* reexam-

ined the OMZ bacteria by high-throughput sequence sampling of all detectable microbial genomes in the water column. The dominant sulfur-oxidizing bacterial lineage was identified as the SUP05 cluster within the γ-proteobacteria, a cosmopolitan cluster of yet-uncultured bacteria that is consistently associated with anaerobic marine water columns (6). The acronym SUP refers to the original discovery of these bacteria in the hydrothermal plume of Suiyo Seamount, a submarine volcano near Japan (7). SUP05 bacteria from this location appear as micrometer-sized coccoid to slightly oval individual free-swimming cells, unattached to particles (see the figure). The Chilean results link the SUP05 lineage with cryptic sulfur cycling and answer the question of why SUP05 sulfide oxidizers are also found in anaerobic, but apparently sulfide-free, waters.

The results also imply that cryptic sulfur cycling and its microbial catalysts are more widespread than currently documented. For example, the SUP05 cluster constituted the most frequently recovered bacterial group in hydrothermal vent plumes at the Mid-Cayman Rise, the deepest currently known hydrothermal vents at 5000 m depth (8).

How resilient is this microbial ecosystem

Department of Marine Sciences, University of North Carolina, Chapel Hill, NC 27599, USA. E-mail: teske@email.unc.edu

in the Chilean OMZ? In sediments with a high input of easily degradable biomass that fuel sulfate reduction, microbial sulfide production can overwhelm the nitrate pool and lead to sulfide poisoning of life in the seabed; sulfide-blackened dead *Thioploca* mats (nicknamed “*Thioploca nigra*”) are a frequent occurrence on the Chilean shelf sediments (9). In the OMZ water column, the chemistry balance is tilted toward sulfide limitation. Comparison of the redox stoichiometries of the pelagic SUP05 group and benthic *Thioploca* lends plausibility to this scenario. Benthic *Thioploca* reduce nitrate to ammonia in order to oxidize sulfide as effectively as possible in a 1:1 ratio of nitrate and sulfide (10). The SUP05 populations favor a more parsimonious mode of sulfide oxidation by the reduction of nitrate to nitrite, with a sulfide/nitrate stoichiometry of 1:4 (see the figure). The dominant OMZ bacteria may have additional, as yet unexplored, mechanisms of

coping with sulfide limitation.

As a caveat, such scenarios oversimplify the very complex nitrogen cycle; they neglect the highly active microbial cycling of ammonia in the oxycline (11). They also do not take into account anaerobic ammonia-oxidizing bacteria in the water column, which combine nitrite and ammonia to nitrogen gas (5). To trace the dynamics of sulfide oxidation and nitrate reduction processes and their microbial populations, Canfield *et al.* call for systematic seasonal studies.

The Chilean OMZ and continental shelf provide two examples for microbial sulfur and nitrogen cycling ecosystems that thrive in a highly dynamic balance and persist through annual or interannual oscillations in redox regime and water column stratification (12, 13). With ocean temperatures and anthropogenic water column anoxia on the rise, nitrate-dependent microbial controls on sulfide concentrations will become increasingly

relevant; in the near future, we will certainly hear more about these resilient microbial engines of the changing world oceans.

#### References

1. D. E. Canfield, A. N. Glazer, P. G. Falkowski, *Science* **330**, 1375 (2010); 10.1126/science.1196889.
2. H. Fossing *et al.*, *Nature* **374**, 713 (1995).
3. V. A. Gallardo, *Nature* **268**, 331 (1977).
4. J. Zopfi, M. E. Bottcher, B. B. Jørgensen, *Geochim. Cosmochim. Acta* **72**, 827 (2008).
5. H. Stevens, O. Ulloa, *Environ. Microbiol.* **10**, 1244 (2008).
6. D. A. Walsh *et al.*, *Science* **326**, 578 (2009).
7. M. Sunamura, Y. Higashi, C. Miyako, J. Ishibashi, A. Maruyama, *Appl. Environ. Microbiol.* **70**, 1190 (2004).
8. C. R. German *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 14020 (2010).
9. V. A. Gallardo, *Gayana Oceanol.* **1**, 27 (1992).
10. S. Otte *et al.*, *Appl. Environ. Microbiol.* **65**, 3148 (1999).
11. V. Molina *et al.*, *Mar. Ecol. Prog. Ser.* **288**, 35 (2005).
12. H. N. Schulz, B. Strotmann, V. A. Gallardo, B. B. Jørgensen, *Mar. Ecol. Prog. Ser.* **200**, 117 (2000).
13. C. E. Morales, S. E. Hormazabal, J. Blanco, *J. Mar. Res.* **57**, 909 (1999).

10.1126/science.1198400

## GENETICS

# The DNA Damage Road Map

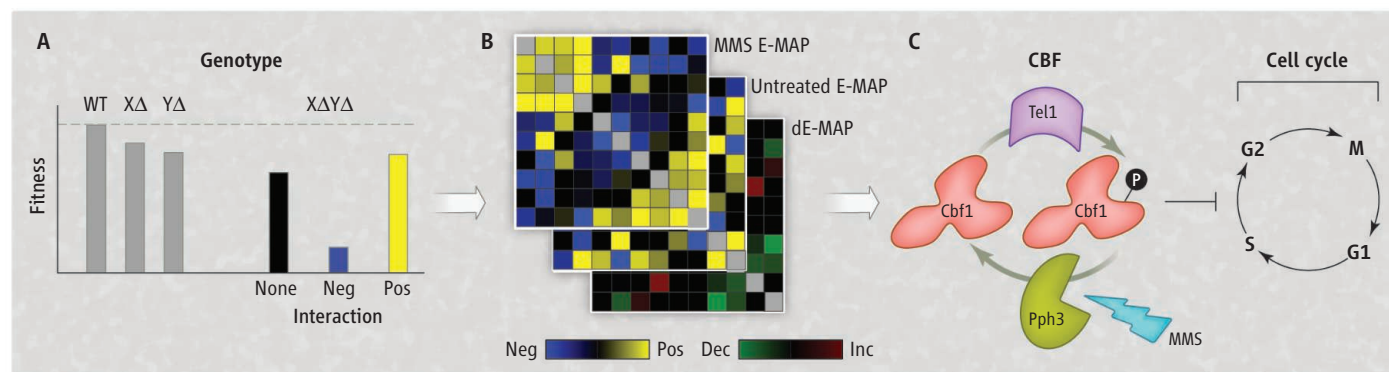
Nir Friedman<sup>1,2</sup> and Maya Schuldiner<sup>3</sup>

If you were on your way to a new country, you would pack a map to help you find the major cities, roads, and interesting places to explore. Recently, researchers have created similar maps to help them start unraveling the complex architecture of

a cell. These maps are created by measuring genetic interactions, specifically the effect that a mutation in one gene has on the phenotype of a mutation in a second gene (see the figure). Using novel genetic tools for studying budding yeast (1) and automated technology, investigators can now systematically and rapidly measure these genetic interactions (epistasis) for all pairs in gene subsets of interest (about 400 to 800 genes). The resulting E-MAPs (epistasis miniarray profiles) (2) have helped chart interactions

Comparing maps of gene interactions offers insight into how yeast cells repair DNA damage.

<sup>1</sup>School of Computer Science and Engineering, The Hebrew University of Jerusalem, Jerusalem, Israel. <sup>2</sup>Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel. <sup>3</sup>Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel. E-mail: nir@cs.huji.ac.il; maya.schuldiner@weizmann.ac.il



**Compare and contrast.** (A) E-MAPs are created by measuring the effect of gene mutations on a phenotype of interest such as fitness. By comparing wild-type (WT) strains to single mutations (XΔ, YΔ) and their combinations (XΔYΔ), investigators can identify mutation pairs that have no effect (black bar, right), a negative effect that is greater than expected (blue bar), or a positive effect that is less

than expected (yellow bar). (B) By comparing E-MAPs of yeast cells grown under treated (MMS) and untreated conditions, researchers created a dE-MAP illustrating the change in interactions (increased or decreased) between the two conditions. (C) The dE-MAP highlighted the role of Tel1 and Pph3 in regulating Cbf1 activity in cell cycle checkpoints (right).



padhyay *et al.* (5) describe the creation of just such a condition-specific E-MAP and a novel method for analyzing it.

Yeast cells can grow under a myriad of conditions by changing their cellular wiring through the regulation of transcription, translation, protein modification, and degradation. To study an example of this changing landscape, Bandyopadhyay *et al.* (5) chose a subset of 418 yeast genes that includes all known signal transduction components and transcription factors, as well as DNA damage proteins and chromatin remodelers. Then, they created two E-MAPs showing how these genes interacted both under standard conditions (“untreated”) and when treated with methyl methanesulfonate (MMS), a DNA-damaging agent (see the figure). A key idea underlying their analysis was to compare and contrast the two maps, thereby isolating interactions that are condition specific (see the figure). They used the differences in these interactions to create a third map, which they named the dE-MAP (differential epistasis mapping). The dE-MAP uncovers novel information on how a yeast cell deals with DNA damage. For example, two genes that encode proteins that repair DNA damage might not show any interaction in untreated cells but might show crucial interactions in yeast treated with MMS.

A bird’s-eye view of the dE-MAP reveals that protein complexes tended to remain stable across the two conditions. The relationships between these complexes, however, were reprogrammed to assist the cell

in dealing with stress. By highlighting these condition-dependent interactions, the dE-MAP allows the scientist interested in DNA damage to zoom in on the regulatory interactions between proteins that play a specific and major role in fighting the effects of MMS. There are hundreds of such stories in the data. One example that demonstrates the power of the approach involves a novel function that the authors found for the transcription factor CBF1 (centromere binding factor) in repressing cell cycle checkpoints (which ensure the fidelity of cell division). One of the advantages of having systematic interaction data are that, instead of examining only specific interactions, E-MAPs allow scientists to examine the “interaction profile” of a gene, or its pattern of interactions with all other genes in the map. This profile provides a rich fingerprint of the gene’s function. Two genes with similar or related functions will have similar interaction profiles. In the past, this concept allowed the identification of several key gene complexes (6). The authors used this tool to reveal that CBF1’s genetic interaction profile changed between the untreated and MMS growth conditions. Only in the MMS E-MAP is CBF1’s activity highly correlated with the activity of two other genes, TEL1 (encoding a kinase) and PPH3 (encoding a phosphatase). Previous work had shown that Tel1 phosphorylates Cbf1, and the authors show that Pph3 regulates CBF1 by dephosphorylating it (see the figure).

With advances in automation and technology, researchers can now easily collect

E-MAPs for many gene subsets and in many conditions. In fact, we are quickly nearing the day when all genetic interactions in yeast will be collected (7). The central challenge has become distilling biological insights from this rich data. This challenge can be met computationally (8–11) or by using careful experimental designs, as shown by Bandyopadhyay *et al.* More broadly, the dE-MAP approach provides an important reminder that most high-throughput interaction data provides just a snapshot representing a specific state. The new frontier is probing the dynamic interactions that enable cells to survive and thrive in varying environmental and genetic contexts. Experiments and analysis at this next, dynamic, level will give biologists truer insights into the ever-changing environment of a living cell.

#### References

1. A. H. Tong *et al.*, *Science* **294**, 2364 (2001).
2. M. Schuldiner *et al.*, *Cell* **123**, 507 (2005).
3. P. Beltrao, G. Cagney, N. J. Krogan, *Cell* **141**, 739 (2010).
4. S. J. Dixon, M. Costanzo, A. Baryshnikova, B. Andrews, C. Boone, *Annu. Rev. Genet.* **43**, 601 (2009).
5. S. Bandyopadhyay *et al.*, *Science* **330**, 1385 (2010).
6. M. Breker, M. Schuldiner, *Mol. Biosyst.* **5**, 1473 (2009).
7. M. Costanzo *et al.*, *Science* **327**, 425 (2010).
8. A. Battle, M. C. Jonikas, P. Walter, J. S. Weissman, D. Koller, *Mol. Syst. Biol.* **6**, 379 (2010).
9. A. Beyer, S. Bandyopadhyay, T. Ideker, *Nat. Rev. Genet.* **8**, 699 (2007).
10. I. Ulitsky, T. Shlomi, M. Kupiec, R. Shamir, *Mol. Syst. Biol.* **4**, 209 (2008).
11. A. Jaimovich, R. Rinott, M. Schuldiner, H. Margalit, N. Friedman, *Bioinformatics* **26**, i228 (2010).

10.1126/science.1199862

## PLANT SCIENCE

# Dynamic Metabolons

Birger Lindberg Møller

The metabolic activities of higher organisms are highly coordinated. At the cellular level, compartmentalization into organelles and substructures thereof optimizes the concentration of substrates targeted by enzymes. At the molecular level, further substrate concentration is gained by the formation of multienzyme complexes—so-called metabolons. The protein constituents of a metabolon are held together by non-covalent interactions and often stabilized by

membrane anchoring. A metabolon allows the direct passage of a product from one enzymatic reaction to a consecutive enzyme in a metabolic pathway. Such channeling of intermediates limits their diffusion into the surrounding milieu, maintains separate pools of intermediates, facilitates fast turnover of labile or toxic intermediates, and may prevent undesired crosstalk between different metabolic pathways (1, 2).

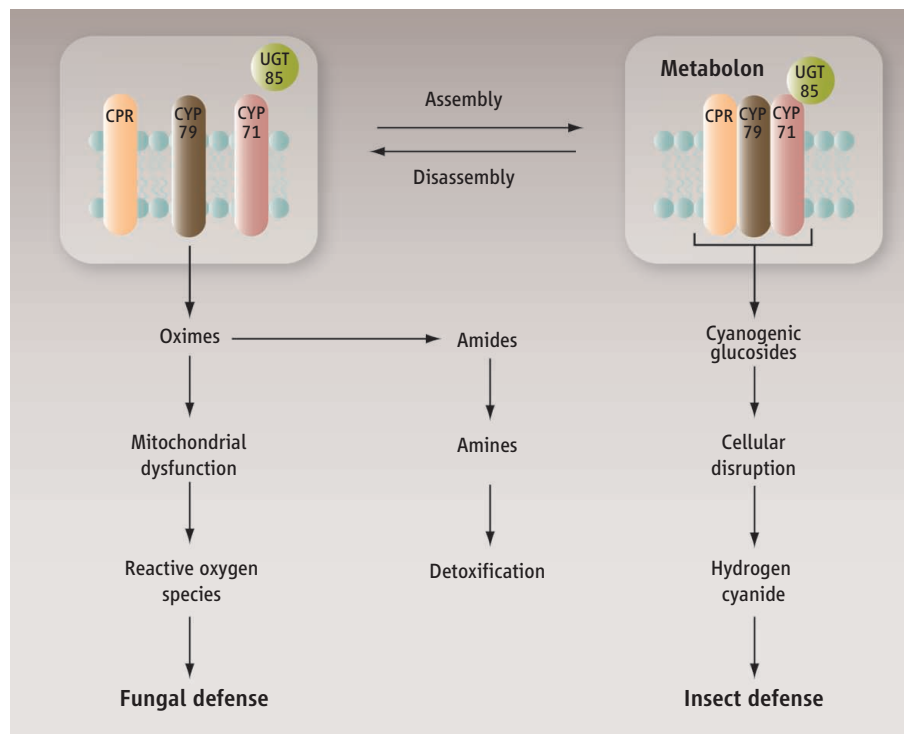
Coordinating metabolic pathways may involve dynamic shifts between the assembly and disassembly of metabolons or their interactions with proteins that modulate their output function. Such transient metabolons are

The assembly and disassembly of enzymes complexes may differentiate plant defense responses to insect attack and fungal infection.

called “functioning dependent structures,” assembling in response to specific metabolic demands or abiotic and biotic challenges (3). For example, in humans, the six enzymes that constitute the purine nucleotide biosynthetic complex assemble upon depletion of purines. Phosphorylation of the metabolon by the CK2 kinase promotes its dissociation, whereas assembly can be initiated by phosphatases or kinase inhibitors (4). The transient complex of glycolytic enzymes that forms at the outer mitochondrial membrane in response to the respiratory demand for pyruvate is another example (5).

In plants, biosynthetic pathways that gen-

Plant Biochemistry Laboratory, Department of Plant Biology and Biotechnology, University of Copenhagen, DK-1871 Frederiksberg C, Copenhagen, Denmark. E-mail: blm@life.ku.dk



**Defense reactions.** Coordinated assembly and disassembly of metabolic complexes, such as the cyanogenic glucoside and glucosinolate metabolons in plants, may optimize the production of chemical constituents that combat pathogens and insects. CPR, cytochrome P450 reductase.

erate bioactive natural products also may be organized as metabolons. This applies to cyanogenic glucosides and glucosinolates (1). Following tissue damage by herbivorous insects, these two classes of phytoanticipins are cleaved by  $\beta$ -glucosidases to release toxic hydrogen cyanide and isothiocyanates, respectively, thereby protecting against generalist insects (6). In both biosynthetic pathways, the initial steps convert amino acids into corresponding oximes, a reaction sequence catalyzed by distinct but homologous cytochrome P450 enzymes of the CYP79 family (7, 8) (see the figure). In cyanogenic glucoside synthesis, the resulting oxime is converted into a labile cyanohydrin by a cytochrome P450 enzyme of the CYP71E subfamily (9), and finally into a stable cyanogenic glucoside by a UGT85 family glucosyltransferase (10). In glucosinolate biosynthesis, the oxime is converted into the final glucosinolate structure via *aci*-nitro compounds or nitrile oxides, *S*-alkylthiohydroximates, thiohydroxamic acids, and desulfoglucosinolates. The intermediates in both synthetic pathways are labile and chemically highly reactive. Metabolon formation may thus facilitate swift conversion and prevent undesired interference with general metabolism.

Numerous pathogenic fungi are not deterred by, or rapidly develop resistance to, hydrogen cyanide and isothiocyanates, and

plants must therefore rely on other defense mechanisms. Recently, a broad-spectrum antifungal defense and innate immune response in plants were proposed to be triggered by the turnover of glucosinolates (11, 12). In this scenario, small amounts of amines were generated, but their route of formation and possible involvement in the innate immune response were not clarified (12). The structures of the amines correspond to those obtained following a Beckmann rearrangement of the oxime intermediates in glucosinolate biosynthesis. One possibility is that amine formation reflects detoxification of oximes that have diffused into noninfected plant cells. Oximes are generally toxic to fungi and are frequently used as chemical fungicides (13, 14). They inhibit mitochondrial oxidases and thereby promote lipid peroxidation and the production of toxic reactive oxygen species (15, 16). The cationic properties of indol-type oximes may increase their toxicity and render them particularly active in launching an innate immune response. Oximes could be formed in plants that produce cyanogenic glucosides and glucosinolates if the respective metabolons were to dissociate in a functioning dependent manner at the precise site of infection. Oximes produced by the released CYP79 enzyme might thus enable preferential combat of fungal infection.

Sorghum produces the cyanogenic glucoside dhurrin. When sorghum microsomes harboring just the two cytochrome P450 enzymes of the metabolon are isolated in the absence of a strong reductant, an oxime is the final product (17). It is not clear what signal would induce dissociation of the metabolon in vivo, but one possibility might be the oxygen burst that accompanies most fungal infections. Conversion of oximes to highly reactive nitroso compounds may also give rise to the formation of diverse protein conjugation products that could signal an innate immune response.

Experimental studies are needed to determine whether oxime production is facilitated by dissociation of the metabolons involved in cyanogenic glucoside and glucosinolate production or by inhibition of the CYP71E enzyme, or whether specific activation of oxime-producing CYP79 enzymes occurs during fungal infection. In all cases, targeted synthesis of highly reactive compounds to combat fungal pathogens would be achieved. Thus, detonation of an “oxime bomb” to combat fungal infection would supplement the ability of cyanogenic glucoside- or glucosinolate-containing plants to detonate a “cyanide bomb” or “mustard oil bomb” when attacked by chewing insects (18).

## References and Notes

1. K. Jørgensen *et al.*, *Curr. Opin. Plant Biol.* **8**, 280 (2005).
2. R. J. Conrado, J. D. Varner, M. P. DeLisa, *Curr. Opin. Biotechnol.* **19**, 492 (2008).
3. M. Thellier, G. Legent, P. Amar, V. Norris, C. Ripoll, *FEBS J.* **273**, 4287 (2006).
4. S. An, M. Kyoung, J. J. Allen, K. M. Shokat, S. J. Benkovic, *J. Biol. Chem.* **285**, 11093 (2010).
5. I. Brandina *et al.*, *Bba-Bioenergetics* **1757**, 1217 (2006).
6. B. L. Møller, *Curr. Opin. Plant Biol.* **13**, 337 (2010).
7. O. Sibbesen, B. Koch, B. A. Halkier, B. L. Møller, *J. Biol. Chem.* **270**, 3506 (1995).
8. U. Wittstock, B. A. Halkier, *J. Biol. Chem.* **275**, 14659 (2000).
9. S. Bak, R. A. Kahn, H. L. Nielsen, B. L. Møller, B. A. Halkier, *Plant Mol. Biol.* **36**, 393 (1998).
10. P. R. Jones, B. L. Møller, P. B. Hoj, *J. Biol. Chem.* **274**, 35483 (1999).
11. N. K. Clay, A. M. Adio, C. Denoux, G. Jander, F. M. Ausubel, *Science* **323**, 95 (2009).
12. P. Bednarek *et al.*, *Science* **323**, 101 (2009).
13. I. Bartosova, K. Kuca, G. Kunesova, D. Jun, *Neurotox. Res.* **9**, 291 (2006).
14. J. E. Drumm *et al.*, *Acc. Sym. Ser.* **584**, 396 (1995).
15. K. Sakurada *et al.*, *Toxicol. Lett.* **189**, 110 (2009).
16. G. M. Rusch, A. Tveit, I. D. Waalkens-Berendsen, A. P. Wolterbeek, G. Armour, *Drug Chem. Toxicol.* **32**, 381 (2009).
17. B. L. Møller, E. E. Conn, *J. Biol. Chem.* **255**, 3049 (1980).
18. A. V. Morant *et al.*, *Phytochemistry* **69**, 1795 (2008).
19. Supported from the Villum Kann Rasmussen research center “Pro-Active Plants”, the UNIK Synthetic Biology Program, and the Danish Research Council for Independent Research.

10.1126/science.1194971



# Opening the Cellular Poison Cabinet

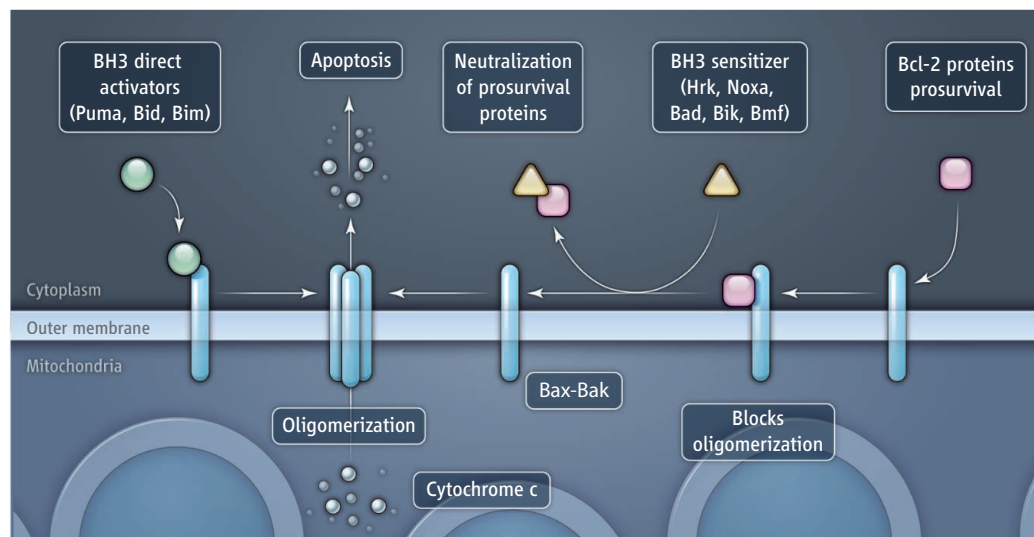
Seamus J. Martin

In complex multicellular organisms, millions of cells die each day as a result of stress, injury, or infection, and as part of the natural cell turnover process that is essential to optimal tissue functioning. Remarkably, cell death in most of these situations is orchestrated by the dying cell itself, a form of cellular suicide called apoptosis (1). On page 1390 in this issue, Ren *et al.* (2) help to resolve how cells execute this process.

A cell must commit suicide at the appropriate time, otherwise malfunctioning or damaged cells could accumulate and lead to tumor development and other pathological conditions. Thus, sensors are needed to monitor the integrity of cellular functions and relay this information to the cell death machinery. A battery of such sensors have been identified—so-called BH3-only proteins (named for the Bcl-2 homology domain 3 they possess)—that detect cellular stress or damage through transcriptional or posttranslational mechanisms (3).

Upon activation, BH3-only proteins provoke permeabilization of the mitochondrial outer membrane, allowing release of cytochrome *c* to the cytosol. This efflux triggers assembly of the apoptosome, a structure that sets in motion a proteolytic cascade that coordinates cell death through destruction of hundreds of proteins (see the figure). Because of their ability to unlock this cellular poison cabinet, BH3-only proteins have come under intensive scrutiny. How do these proteins provoke cytochrome *c* efflux?

Bax and Bak, proteins that either reside in or insert into mitochondrial membranes, constitute the pore or channel that permeabilizes mitochondria. Loss of Bax and Bak renders cells resistant to permeabilization (and subsequent apoptosis) caused by BH3-only proteins (4). Upon activation, BH3-only proteins promote oligomerization of Bax and Bak within the mitochondrial outer membrane. Thus, opening of the resultant



**Death decisions.** Bax–Bak–dependent mitochondrial outer membrane permeabilization is positively regulated through the actions of BH3-only sensors and negatively regulated through pro-survival Bcl-2 family proteins.

channel effectively constitutes the decision to commit cellular suicide.

Because of the deadly consequences of mitochondrial outer membrane permeabilization, this major checkpoint on the road to cell death is heavily policed. Opening of the Bax–Bak channel is opposed by the pro-survival members of the Bcl-2 family (Bcl-2, Bcl-x, Mcl-1, A1, Bcl-w, and Bcl-b). These proteins either bind to Bax and Bak and prevent oligomerization, or are “neutralized” when they are directly bound by BH3-only proteins. Thus, BH3-only proteins and pro-survival Bcl-2 proteins represent opposing forces in the struggle to control the channel, the outcome of which dictates whether a cell will live or die. This has led to two alternative models, not necessarily mutually exclusive, to explain how BH3-only proteins promote mitochondrial outer membrane permeabilization (5, 6).

The “sensitizer model” argues that BH3-only proteins act indirectly to promote cell death by binding to pro-survival Bcl-2 proteins. This interaction liberates Bax and Bak from their pro-survival captors and allows oligomerization and channel formation (6). The “direct activation” model argues that at least some BH3-only proteins directly bind to Bax and Bak and promote the conformational changes that allow oligomerization. Indeed, only a subset of BH3-only proteins

The assembly and activation of a mitochondrial channel is triggered by direct interaction with signaling molecules that promote cell death.

(Bid, Bim, and Puma) directly activates Bax and Bak, whereas the remaining BH3-only proteins neutralize pro-survival Bcl-2 proteins (5).

The direct activation model predicts that loss of the direct activators (Bid, Bim, and Puma) renders the Bax–Bak channel incapable of activation, thereby producing cells that are resistant to apoptotic signals. By contrast, the sensitizer model predicts that cells lacking this subset of BH3-only proteins can still undergo mitochondrial permeabilization through the remaining BH3-only proteins. Ren *et al.* support the direct activation model by demonstrating that deletion of the *Bim*, *Bid*, and *Puma* genes in mice produces defects very similar to those displayed by mice lacking the *Bax* and *Bak* genes, rendering cells refractory to Bax–Bak activation in response to apoptotic stimuli. Mice deficient in the three putative direct activators (Bid, Bim, and Puma) displayed developmental defects (interdigital webs, imperforate vaginas, and enlarged lymphocyte populations) and were born at 50% of the expected Mendelian frequency, indicating partial embryonic lethality. Biochemical evidence also suggests that certain BH3-only proteins act as direct Bax–Bak activators (7, 8), although these interactions are transient and hard to record with conventional techniques. Thus, accumulat-

ing evidence argues that a subset of BH3-only proteins function as direct activators of Bax and Bak, and indeed, may be indispensable for this. The work of Ren *et al.* provides strong support for the idea that Bim, Bid, and Puma are the central Bax-Bak activators, with the remaining BH3-only proteins functioning primarily as sensitizers. The major distinction between these two categories of cell death sensors may be their relative affinities for Bax-Bak compared to those of the prosurvival Bcl-2 proteins. The emerging view is that a combination model of activator-sensitizer appears to most accurately reflect BH3-only protein function.

The precise composition and structure of the Bax-Bak channel have yet to be elucidated, and how BH3-only proteins sense the multitude of signals that trigger apoptosis is not yet fully understood. However, there is progress toward developing targeted thera-

pies aimed at selectively engaging the Bax-Bak channel in cell populations that resist death due to disturbances among the ranks of BH3-only proteins and their prosurvival Bcl-2 family antagonists (9). In the case of tumors that adopt the strategy of survival by avoiding cell death, direct activator BH3-only mimetic drugs may prove highly effective antidotes.

#### References

1. R. C. Taylor, S. P. Cullen, S. J. Martin, *Nat. Rev. Mol. Cell Biol.* **9**, 231 (2008).
2. D. Ren *et al.*, *Science* **330**, 1390 (2010).
3. R. J. Youle, A. Strasser, *Nat. Rev. Mol. Cell Biol.* **9**, 47 (2008).
4. T. Lindsten *et al.*, *Mol. Cell* **6**, 1389 (2000).
5. T. Kuwana *et al.*, *Mol. Cell* **17**, 525 (2005).
6. S. N. Willis *et al.*, *Science* **315**, 856 (2007).
7. P. F. Cartron *et al.*, *Mol. Cell* **16**, 807 (2004).
8. J. F. Lovell *et al.*, *Cell* **135**, 1074 (2008).
9. T. Oltschdorf *et al.*, *Nature* **435**, 677 (2005).

10.1126/science.1199461

## NEUROSCIENCE

# Dedicated to Memory?

Howard Eichenbaum

**Rats with a damaged perirhinal cortex exhibit false memory, raising questions about brain organization.**

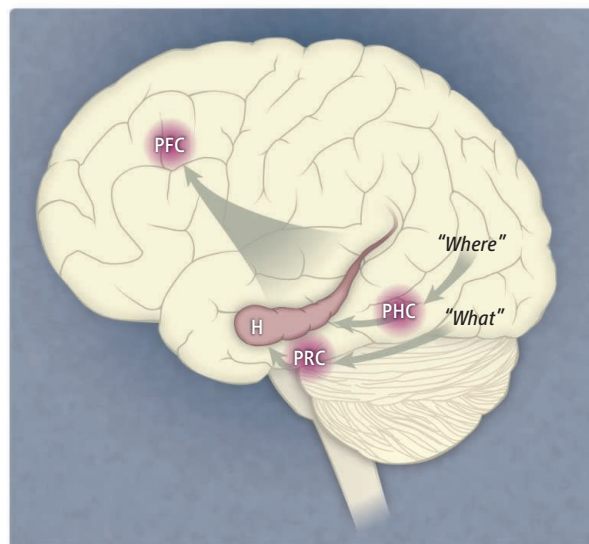
Early observations of individuals with circumscribed damage to the cerebral cortex led to a consensus that memory is not localized to any particular brain area. Rather, neuroscientists believed that memories were incorporated within the information processing functions of many specialized brain areas. In 1957, this view changed dramatically after Scoville and Milner (1) described a patient, known as H.M., in whom damage to the medial temporal lobe (MTL; including the hippocampus and surrounding cortex) resulted in global memory impairment but spared perceptual and cognitive functions. A principal interpretation of these findings was that the MTL is a dedicated memory system, and this perspective dominated subsequent research on memory (2). Recent studies have called this idea into question, however, and McTighe *et al.* (3) add another twist on page 1408 of this issue. They claim that one part of the MTL, the perirhinal cortex, has a specific information processing function not directly related to memory. Does this finding turn the clock

back to the dedicated area view, or move it forward in understanding how the MTL memory system is organized?

McTighe *et al.* employed a clever variation of a popular memory-research paradigm. In the usual procedure, researchers initially present a rat with a novel object for a few minutes and then, after a significant

delay period, simultaneously present the rat with two test objects: the now “familiar” one and a new “novel” object. Typically, rats that do not have brain damage spend less time exploring the familiar object (demonstrating memory). Rats with perirhinal cortex damage spend about the same amount of time exploring both objects, suggesting that they have forgotten the familiar object. In McTighe *et al.*’s variation, they presented the familiar and novel test objects separately, instead of simultaneously. They showed that animals with perirhinal damage did not forget the familiar object but rather treated the novel object as familiar, a kind of false memory. On the basis of this and previous findings, the authors argue that the perirhinal cortex’s normal role in recognition is to configure, or bind, elemental features of stimuli already processed earlier in the perceptual pathway. If the perirhinal cortex is damaged, however, object memories exist only as fragmented representations of these featural elements formed at the earlier stages. These fragmented memories are susceptible to interference from a constant natural stream of subsequent perceptual input, leading to the false memory for test items that share features with the interfering inputs. Consistent with this idea, McTighe *et al.* were able to eliminate false memories in brain-damaged rats by placing the animals into a dark chamber during the delay, thus reducing new perceptual interference.

How do these findings affect the dedicated memory system view? McTighe *et al.* support a return to the idea that brain areas contribute to memory only as a by-product of their specialized information processing functions, in this case the perirhinal cortex’s perceptual binding function. However,



**Functional organization of a memory system.** Information about specific objects and events arrives through the “what” cortical stream into perirhinal cortex (PRC), while information about the spatial-temporal context in which events occurred arrives through the “where” cortical stream into parahippocampal cortex (PHC). These two streams of information combine within the hippocampus (H), which represents relationships between objects and events and their context. When cued for a memory, feedback pathways from the hippocampus to the cortical areas generate representations of object and context memories that are tested for a match in prefrontal cortex (PFC).



other recent studies that distinguish between forgetting and false memories due to damage in other parts of the MTL system offer a broader perspective. In these experiments, rats initially study items chosen from a large list each day, and then investigators measure their memory performance in terms of “hits” (correct identifications of stimuli that were on that study list) and “false alarms” (errors where subjects incorrectly judge new stimuli as appearing on the study list). Damage to the hippocampus results in a decrease in hits with no effect on the false alarm rate (4), indicating that the deficit is due to forgetting rather than false memories—the opposite of the pattern observed by McTighe *et al.* In contrast, damage to the prefrontal cortex results in an increase in false alarms and no effect on the hit rate (5), similar to the pattern observed by McTighe *et al.* These studies suggest that distinguishing forgetting from false memory provides researchers with a powerful tool for identifying the contributions of different brain areas to memory.

Furthermore, these diverse findings can be integrated into a model of the anatomical pathways of MTL system and its interactions with cortical areas (see the figure) (6). The perirhinal cortex is the end point of the so-called “what” stream, a cortical hierarchy of perceptual processing areas that represents information about objects (7). There is also

a parallel “where” stream in the cortex, ending in the MTL within the parahippocampal area, which represents the spatiotemporal contexts in which objects have been experienced. These streams merge within the hippocampus, which represents relationships between objects and between objects and their context. In this model, the role of the perirhinal cortex is to bind perceptual features into representations of whole objects, and make these representations resistant to perceptual interference—just as McTighe *et al.* describe. The hippocampus normally encodes and retrieves representations of the objects and context when cued, and damage to the hippocampus prevents this retrieval, resulting in forgetting. The prefrontal cortex normally receives that information from feedback pathways through the MTL, and monitors the match between object and context memories (“Is this object from today’s list?”). Lacking that monitor, the animals cannot tell whether an object is from today’s list or a previous list—leading to the increase in false memories following prefrontal damage.

A decade ago (2), available evidence led to the conclusion that different forms of memory should be viewed as the outcome of plasticity within systems organized to perform particular information processing functions. The MTL’s information processing functions, however, were unclear. In their

study, McTighe *et al.* offer a partial answer: The MTL’s perirhinal cortex binds featural elements into cohesive configural memories, and this function is supported by known plasticity mechanisms (8). The hippocampus and prefrontal cortex, as well as other key brain areas of this system, also contribute directly to memory through plasticity in their particular information processing tasks. These findings, then, support the view that there is a dedicated MTL memory system. They also further our understanding of that system as a set of specialized areas that interact to coordinate the information processing functions required for successful memory.

#### References

1. W. B. Scoville, B. Milner, *J. Neurol. Neurosurg. Psychiatry* **20**, 11 (1957).
2. H. Eichenbaum, N. J. Cohen, *From Conditioning to Conscious Recollection: Memory Systems of the Brain* (Oxford Univ. Press, New York, 2001).
3. S. M. McTighe, R. A. Cowell, B. D. Winters, T. J. Bussey, L. M. Saksida, *Science* **330**, 1408 (2010).
4. N. J. Fortin, S. P. Wright, H. Eichenbaum, *Nature* **431**, 188 (2004).
5. A. Farovik, L. M. Dupont, M. Arce, H. Eichenbaum, *J. Neurosci.* **28**, 13428 (2008).
6. H. Eichenbaum, A. P. Yonelinas, C. Ranganath, *Annu. Rev. Neurosci.* **30**, 123 (2007).
7. L. G. Underleider, M. Mishkin, in *Two Cortical Visual Systems. Analysis of Visual Behavior*, M. A. Ingle, M. I. Goodale, R. J. W. Masfield, Eds. (MIT Press, Cambridge, MA, 1982), pp 549–586.
8. S. Griffiths *et al.*, *Neuron* **58**, 186 (2008).

10.1126/science.1199462

## MATERIALS SCIENCE

# High-Temperature Rubber Made from Carbon Nanotubes

Yury Gogotsi

Carbon nanotubes have been among the most studied materials for the past two decades (1); they display several remarkable properties, such as extremely high tensile strength and electrical conductivity. On page 1364 of this issue, Xu *et al.* (2) report another case of extreme mechanical performance of a carbon material—viscoelastic behavior of nanotubes in a wide temperature range—that no other solid has shown so far.

Viscoelasticity is the ability of a material to dissipate energy through viscous behavior

(think honey) and reversibly deform through elasticity (think rubber band). Polymer foam earplugs are a typical viscoelastic material; they conform to any shape of ear channel but fully recover to the original form after being pulled out. Viscoelasticity is exhibited by a large number of materials (3), including amorphous and semicrystalline polymers, biomaterials, crystalline materials experiencing reversible phase transformations, and some metallic alloys.

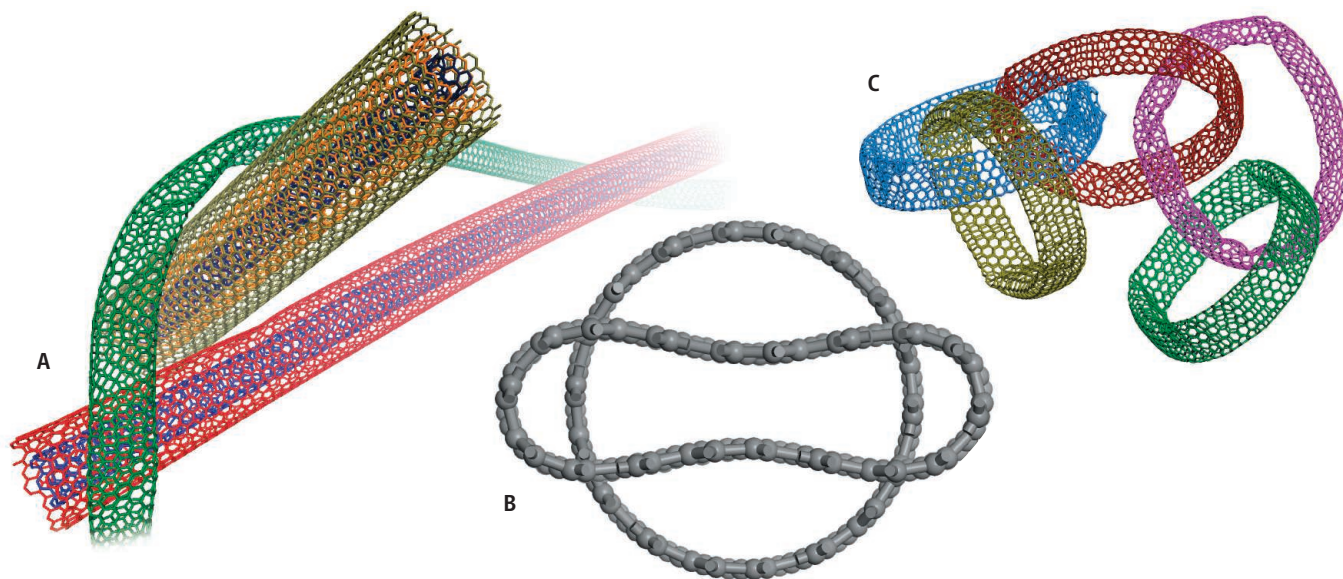
Viscoelastic behavior is determined by measuring stress-strain curves: The material is pushed on or pulled at a given force (stressed), and deformation (strain) is measured. A viscoelastic material exhibits “memory” (hysteresis) effects in its stress-

strain behavior. For example, the amount of stress needed to maintain the same level of strain will drop over time, and for a given stress, the material will continue to deform.

The material reported by Xu *et al.* is a special case of a viscoelastic material; it behaves like rubber under moderate deformations. Rather than store energy in permanent deformation, like a bent metal part, a rubber releases the energy when the applied force is removed. Viscoelastic behavior of nanotubes has been observed for vertically aligned brushes and foams of tubes (highly intertwined random networks) tested at room temperature (4–7). The groups of Gogotsi and Greer independently observed buckling and irreversible compressibility

A mixture of carbon nanotubes creates a material that can recover its shape after deformation over a wide temperature range.

Department of Materials Science and Engineering, Drexel University, Philadelphia, PA 19104, USA. E-mail: gogotsi@drexel.edu



**Nanotube arrangements leading to rubbery materials.** Images are from atomistic models of the displacement of entangled nanotubes relative to each other or temporary collapse (flattening) of thin tubes. (A) Three nanotubes (single-walled, double-walled, and triple-walled) are shown in contact with each other and interacting only by van der Waals forces. (B) The dumbbell shape of the collapsed nanotube decreases the distance between the walls to the interplanar spacing of graphite and temporarily creates van der Waals bonding in the middle area of the tube. (C) A hypothetical material consisting of interlocking nanotube rings that would be expected to display viscoelastic behavior.

of various nanotube architectures at large strains (4, 5). The material developed by Xu *et al.* is a random network of long, interconnected and entangled carbon nanotubes (a mix of single-, double-, and triple-walled tubes) that exhibits rubberlike behavior over a very wide temperature range. They tested their samples from  $-196^{\circ}\text{C}$  to  $1000^{\circ}\text{C}$ , but the carbon-carbon bonds in graphitic walls of nanotubes are stable above  $1500^{\circ}\text{C}$ . Thus, an even broader temperature range can be expected for viscoelastic response, at least in a non-oxidizing environment. The demonstration of viscoelastic behavior and large reversible deformation for a wide temperature range makes the random, entangled nanotube network described by Xu *et al.* a versatile viscoelastic material. It can dampen vibrations and absorb impact at extremely low or very high temperatures, where neither a perfectly elastic rubber nor a metallic felt or wool would work.

The viscoelastic behavior of the most typical viscoelastic materials, such as polymers, is temperature-dependent; the molecular rearrangement of polymer chains, a

mechanism governing viscoelasticity, is a thermally activated process. Xu *et al.* suggest that, unlike polymers, thermal stability in their material stems from energy dissipation through the zipping and unzipping of carbon nanotubes upon contact (see the figure, panel A). An additional mechanism of energy dissipation is the flattening and recovery of nanotubes (see the figure, panel B). For the tubes with inner diameters of 3 to 5.5 nm, the collapsed state is metastable and is separated from the energetically favorable cylindrical shape by an energy barrier (8). The barrier decreases with the diameter and disappears when the single-walled tube diameter is smaller than 3 nm.

The entangled nanotube material is a kind of versatile rubber that could be used in cold interstellar space or inside a high-temperature vacuum furnace. It may not be easy to find a material with viscoelastic properties similar or superior to those reported by Xu *et al.* Thin-walled carbon nanocapsules (onions or giant multishell fullerenes) may offer reversible compressibility (9) similar to nanotubes (see the figure, panel B), but only fibrous materials can offer rubberlike behavior and such a large reversible deformation under tension. Graphene foam would lack elastic recovery because there would not be a driving force necessary to accommodate it; moreover, all carbon foams and porous carbons—even those that show plasticity and elastic recovery at the nano- and microscale (10)—fail in brittle manner when subjected to macroscopic deformation. Nanotube rings may form an incompressible material showing rubberlike behavior, but such an arrangement of carbon rings (see the figure, panel C) remains hypothetical.

Many high-temperature applications of materials expose them to oxidizing conditions. Most small-diameter nanotubes burn at temperatures close to  $400^{\circ}\text{C}$ , so very-high-temperature applications may only be possible in a vacuum or protective (reducing) atmosphere. Incorporating these nanotube rubbers into commercial products will depend in part on lowering of material costs, but note that the cost and production volume issues have been largely resolved for multiwalled tubes that are at least 5 nm in diameter. With further developments, nanotube materials may find use not only in space vehicles but also in down-to-earth applications, such as wrinkle-free textiles or viscoelastic shoe insoles that reduce mechanical shocks.

#### References and Notes

1. R. H. Baughman, A. A. Zakhidov, W. A. de Heer, *Science* **297**, 787 (2002).
2. M. Xu, D. N. Futaba, T. Yamada, M. Yumura, K. Hata, *Science* **330**, 1364 (2010).
3. R. S. Lakes, *Viscoelastic Materials* (Cambridge Univ. Press, Cambridge, 2009).
4. S. Pathak, Z. G. Cambaz, S. R. Kalidindi, J. G. Swadener, Y. Gogotsi, *Carbon* **47**, 1969 (2009).
5. S. B. Hutchens, L. J. Hall, J. R. Greer, *Adv. Funct. Mater.* **20**, 2338 (2010).
6. A. Cao, P. L. Dickrell, W. G. Sawyer, M. N. Ghasemi-Nejhad, P. M. Ajayan, *Science* **310**, 1307 (2005).
7. Q. Zhang *et al.*, *J. Phys. D* **43**, 315401 (2010).
8. S. Rotkin, Y. Gogotsi, *Mater. Res. Innov.* **5**, 191 (2002).
9. K. Asaka, K. Miyazawa, T. Kizuka, *Nanotechnology* **20**, 385705 (2009).
10. Y. Gogotsi, *Carbon Nanomaterials* (CRC Press, Boca Raton, FL, 2006).
11. I thank V. Mochalin for Material Studio simulation of viscoelastic nanotube structures, J. R. Greer for helpful comments, and the U.S. Department of Energy, Office of Basic Energy Sciences, for research funding.

10.1126/science.1198982



# A New Approach to Fluorescence Microscopy

Mark Bates

The fluorescence microscope is a powerful tool for the study of molecular and cell biology, enabling researchers to peer into cells and living organisms to understand their spatial organization. Methods for tagging specific biomolecules with fluorescent labels, such as green fluorescent protein (GFP), provide a toolbox for the observation of protein organization, interactions, and dynamics (1). This enables the visualization of a host of biological phenomena, such as enzymatic processes, the development of cells and organisms, and the structural dynamics of individual biomolecules.

Despite its benefits, a significant drawback of fluorescence microscopy is its spatial resolution. Beyond a certain magnification, the fine structure of a specimen is obscured when viewed through the optical microscope. As described by Abbe in the late 19th century, the diffraction of light imposes a resolution limit, which makes it impossible to resolve spatial features smaller than about 250 nm using visible light. This leaves much of the architecture of cells and their inner workings inaccessible to study. The electron microscope allows researchers to obtain images with higher resolution, but in this case the sample preparation is not compatible with live cell imaging, and it is difficult to achieve the molecular specificity and multicolor capability offered by fluorescent labeling.

My graduate thesis focused on the development of an imaging modality that would combine the advantages of both electron microscopy and fluorescence imaging. In the past several years, a number of innovative approaches have been conceived to capture fluorescence images with nanometer-scale spatial resolution (2, 3). These techniques take advantage of nonlinearities inherent in molecular fluorescence, such as saturation or depletion of the excited state, to obtain optical resolutions an order of magnitude beyond the classical diffraction limit. We invented a new approach that is based on single-molecule detection and localization, using a standard fluorescence microscope and readily available fluorescent labels, for wide appli-

cability to the life sciences.

Early in my doctoral research, I made a surprising observation about a commonly used red fluorophore, Cy5: Its fluorescence emission can be switched on and off using pulses of light. We studied this "optical switching" effect and found that during each excitation, Cy5 emits thousands of photons before going dark. A brief pulse of ultraviolet light will then efficiently reactivate the molecule to its fluorescent state, and this process can be repeated for hundreds of cycles (4, 5). We realized that the switchable fluorescence exhibited by Cy5 is a strongly nonlinear process, and we reasoned that this nonlinearity could be used to overcome the diffraction limit of resolution.

Inspired by this idea, we found a novel method to obtain sub-diffraction limit fluorescence images (see the figure, parts A to D). If a biological sample is labeled with photoswitchable fluorophores, the experiment can be controlled so that only one fluorophore, or a sparse subset of fluorophores, are in the fluorescent state at any given time. The image of each isolated fluorophore

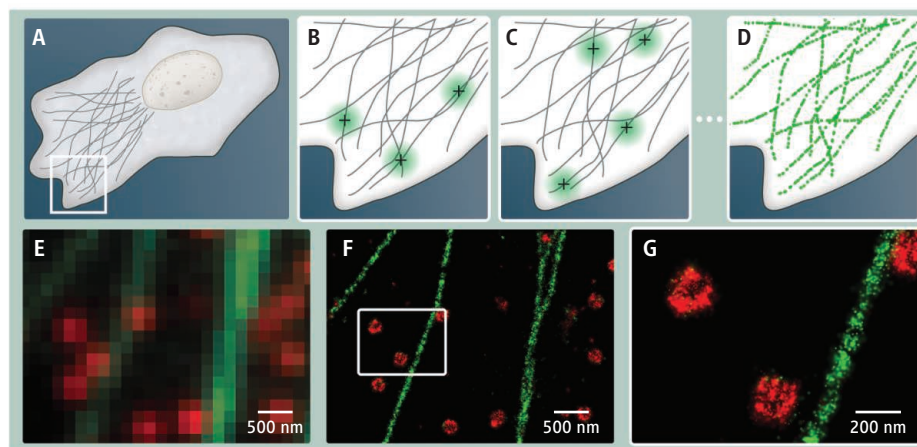
Precise localization of switchable fluorescent molecules facilitates nanoscale biological imaging.

GE Healthcare and *Science* are pleased to present the prize-winning essay by Mark Bates, a regional winner from North America, who is the Grand Prize winner of the GE & *Science* Prize for Young Life Scientists.



appears as a diffraction-limited spot several hundred nanometers in size. By measuring the centroid of this spot, the position of the fluorophore can be determined with a high degree of precision, often on the nanometer scale (6, 7). When the active fluorophores have been switched off, a new subset is stochastically activated by a pulse of activation light, and the process is repeated. When a sufficient number of fluorophore positions have been obtained, a plot of their coordinates reconstructs an image of the sample. The spatial resolution of the resulting image is not diffraction-limited. Rather, it is limited only by the precision of each fluorophore localization and the number of localizations present. We called this method stochastic optical reconstruction microscopy, or STORM (8). This concept for imaging was simultaneously developed by several groups, and it is also known as PALM and fPALM (9, 10).

Multicolor fluorescence imaging enables the study of the relative organization and interactions of cellular components, and we developed a strategy for multicolor STORM using Cy5 as the photoswitchable fluorophore. We found that when Cy5 is paired



**STORM imaging.** (A to D) A cell labeled with switchable fluorophores (A) is imaged by activating only a subset of fluorophores at a time (B and C). The locations of each fluorophore are determined computationally (crosses) and a plot of their coordinates yields a super-resolution image which is not limited by diffraction (D) (17). A comparison of conventional epifluorescence imaging (E) and the corresponding STORM image (F) of clathrin-coated pits (red) and microtubules (green). The boxed region in (F) is magnified in (G).

The author is in the Department of NanoBiophotonics, Max Planck Institute for Biophysical Chemistry, 37077 Göttingen, Germany. E-mail: mbates@ggweg.de.

with a second fluorophore (such as Cy2 or Cy3), its activation wavelength is strongly influenced by the spectral characteristics of its neighbor (11). We took advantage of this property to selectively activate specific populations of dye pairs, enabling us to capture images of multiple molecular targets within the same sample. Using antibodies labeled with either Cy3 and Cy5, or Cy2 and Cy5, we imaged microtubules and clathrin-coated pits in cultured mammalian cells (see the figure, parts E to G). In comparison with the conventional fluorescence image, the STORM image reveals previously unseen detail due to its high spatial resolution (~25 nm) and clearly resolves the rounded structure of clathrin pits only 150 to 200 nm in diameter (11).

Many cellular structures have a complex three-dimensional (3D) shape, requiring a 3D imaging method for study. Taking advantage of a technique for 3D particle localization, we used astigmatic imaging to generate 3D STORM images with 25-nm lateral resolution and 50-nm axial resolution. With this strategy, we were able to obtain images of the full spherical structure of clathrin-coated pits (12).

Although imaging techniques based on this concept are relatively recent, they have already been used to understand mechanisms in biology. Greenfield *et al.* (13) have used these techniques to develop a model of how chemotaxis receptors in *Escherichia coli* organize in growing cells. Biteen *et al.* (14) have visualized the nanoscale structure of MreB in live *Caulobacter crescentus*, taking advantage of the photoswitchable fluorescence of enhanced yellow fluorescent protein (EYFP). Also, Hess *et al.* (15) have obtained high-resolution images and dynamic information from influenza hemagglutinin, a clustered membrane protein, to differentiate between membrane organization models in fibroblast cells.

The development of sub-diffraction limit fluorescence microscopy has created new possibilities for the observation of biological processes, and a new assay for the organization and composition of biomolecular complexes. With continued advances in fluorescent labels and labeling methods (16), it will be exciting to see how these techniques are applied to bring about insights into life at the nanometer scale.

## References and Notes

1. B. N. Giepmans, S. R. Adams, M. H. Ellisman, R. Y. Tsien, *Science* **312**, 217 (2006).
2. S. W. Hell, *Science* **316**, 1153 (2007).
3. M. G. L. Gustafsson, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 13081 (2005).
4. M. Bates, T. R. Blosser, X. Zhuang, *Phys. Rev. Lett.* **94**, 108101 (2005).
5. G. T. Dempsey *et al.*, *J. Am. Chem. Soc.* **131**, 18192 (2009).
6. R. E. Thompson, D. R. Larson, W. W. Webb, *Biophys. J.* **82**, 2775 (2002).
7. A. Yildiz *et al.*, *Science* **300**, 2061 (2003).
8. M. J. Rust, M. Bates, X. Zhuang, *Nat. Methods* **3**, 793 (2006).
9. E. Betzig *et al.*, *Science* **313**, 1642 (2006).
10. S. T. Hess, T. P. Girirajan, M. D. Mason, *Biophys. J.* **91**, 4258 (2006).
11. M. Bates, B. Huang, G. T. Dempsey, X. Zhuang, *Science* **317**, 1749 (2007).
12. B. Huang, W. Wang, M. Bates, X. Zhuang, *Science* **319**, 810 (2008).
13. D. Greenfield *et al.*, *PLoS Biol* **7**, e1000137 (2009).
14. J. S. Biteen *et al.*, *Nat. Methods* **5**, 947 (2008).
15. S. T. Hess *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 17370 (2007).
16. M. Fernandez-Suarez, A. Y. Ting, *Nat. Rev. Mol. Cell Biol.* **9**, 929 (2008).
17. Figure panels A to D are adapted from *Current Opinion in Chemical Biology*, vol. 12, "Super-resolution microscopy by nanoscale localization of photo-switchable fluorescent probes," M. Bates, B. Huang, and X. Zhuang, pages 505–514, Copyright 2008, with permission from Elsevier.

10.1126/science.1200252

## 2010 Grand Prize Winner



**Mark Bates** was born in Toronto, Canada. He received a B.Sc. degree in engineering physics from Queen's University and an M.Sc. degree in physics from McGill University. He conducted his doctoral research at Harvard University, working under the guidance of Xiaowei Zhuang, where he studied the properties of photoswitchable fluorescent molecules and applied these results to develop a new method for high-resolution optical imaging. Dr. Bates is

now a postdoctoral fellow in the laboratory of Stefan Hell in Göttingen, Germany, where he is applying super-resolution fluorescence microscopy to study prokaryotic cell biology.

### Regional Winners

**Europe:** Ataman Sendoel for his essay "Is Death Without Oxygen as Sweet as Apoptosis?" Dr. Sendoel was born in Zurich, Switzerland. He studied medicine at the Universities of Zurich and Lausanne. After finishing medical school, he joined the M.D.-Ph.D. program of the University of Zurich. He conducted his Ph.D. work in the laboratory of Michael Hengartner, where he studied mechanisms of controlling programmed cell death in *Caenorhabditis elegans*. Dr. Sendoel is currently a postdoctoral fellow and continues to work on hypoxia responses in *C. elegans*.



**Japan:** Sakiko Honjoh for her essay "Is Aging Necessary?" Dr. Honjoh was born in Yokohama, Japan. Inspired by a high-school biology teacher, she decided to major in molecular biology and entered Kyoto University. Continuing on this track, Dr. Honjoh completed her Ph.D. in the laboratory of Eisuke Nishida at the Graduate School of Biostudies, Kyoto University, working on the signal transduction networks that regulate life span. She is continuing her work in the same lab, still trying to elucidate the molecular changes that occur during aging.

**All Other Countries:** Melissa Fullwood for her essay "Genome-Wide Chromatin Loops Regulate Transcription." Dr. Fullwood, born and raised in Singapore, graduated from Stanford University in 2005 and completed her Ph.D. in 2009 at the Genome Institute of Singapore under the auspices of the National University of Singapore where she was supervised by Yijun Ruan. In 2009, she was selected for the inaugural L'Oreal for Women in Science National Fellowships in Singapore. She is currently a Lee Kuan Yew Post-Doctoral Fellow at the Duke-NUS Graduate Medical School Singapore under the supervision of Shirish Shenolikar.



For the full text of essays by the regional winners and for information about applying for next year's awards, see *Science Online* at [www.sciencemag.org/feature/data/prizes/ge/index.dtl](http://www.sciencemag.org/feature/data/prizes/ge/index.dtl).





## INTRODUCTION

# Metabolism Is Not Boring

SO INVESTIGATOR STEVEN MCKNIGHT ADMONISHED HIS AUDIENCE LAST SPRING after a presentation at a conference (on metabolism and cancer, no less). He may have been “preaching to the choir” in this case, but what led him to issue such a blunt reminder? It seems that for a generation of biological scientists, metabolism was an area of biochemistry to be mastered and then put aside. Metabolism was always there in the background, providing the cell with the energy and resources to do what was required, but was rarely recognized to determinedly influence, and be influenced by, the physiological state of the cell.

This special issue of *Science* celebrates a resurgence of interest in metabolism and an appreciation of its central role in disparate areas of cell biology, physiology, medicine, and synthetic biology ([www.sciencemag.org/special/metabolism/](http://www.sciencemag.org/special/metabolism/)). McKnight's Perspective (p. 1338) takes a broad look at the field and justifies his excitement that recognizing the reciprocal regulation of metabolism and other cellular processes promises to advance our understanding of complex physiology.

There is renewed interest in the metabolism of cancer cells and its potential as a therapeutic target. Levine and Puzio-Kuter (p. 1340) review metabolic changes in cancer cells, as well as the recent suggestion that alterations in a metabolic enzyme can lead to the production of an “oncometabolite” that supports cancer cell growth. When energy sources are limited, cells use a process known as autophagy for breaking down cellular components to provide substrates for metabolism. Rabinowitz and White's Review (p. 1344) discusses roles of autophagy in metabolism, its regulation, and its implications for cancer and degenerative diseases.

Of course not all fields have neglected metabolism over the years, and an enormous literature describes the role of insulin and related hormones in controlling cellular metabolism. In a Focus Issue of *Science Signaling*, a Research Article and Perspective describe an unexpected signal from the insulin receptor that confers sensitivity to cell death when insulin is not present. Also highlighted are a role for lipids as cellular sensors of glucose metabolism and a mechanism by which cells can survive oxidative stress by shifting the activity of the cell's protein degradation complex, the proteasome.

In *Science Translational Medicine*, Vallerie and Hotamisligil review how in a strategy to combat obesity and insulin resistance, a therapeutic inhibitor of cell signaling pathways must have coordinated actions in multiple cell types. Back in *Science*, Bass and Takahashi (p. 1349) review new insights into the interaction of metabolism with circadian clocks. Displacement of eating and periods of activity away from the normal light-dark cycle, as experienced in jet lag or shift work, have marked effects on metabolic diseases.

Cellular metabolic pathways, particularly in yeast or bacteria, can be exploited to synthesize compounds that are difficult or expensive to produce by other means. Keasling (p. 1355) reviews advances in metabolic engineering and looks forward to a future in which customized microbes made by computer-aided design can efficiently produce desired chemicals, ranging from fuels to pharmaceuticals.

See? It's not boring at all!

— L. BRYAN RAY

## Metabolism

### CONTENTS

#### Perspective

- 1338 On Getting There from Here  
S. L. McKnight

#### Reviews

- 1340 The Control of the Metabolic Switch in Cancers by Oncogenes and Tumor Suppressor Genes  
A. J. Levine and A. M. Puzio-Kuter
- 1344 Autophagy and Metabolism  
J. D. Rabinowitz and E. White
- 1349 Circadian Integration of Metabolism and Energetics  
J. Bass and J. S. Takahashi
- 1355 Manufacturing Molecules Through Metabolic Engineering  
J. D. Keasling

See also *Science Translational Medicine* and *Science Signaling* at [www.sciencemag.org/special/metabolism/](http://www.sciencemag.org/special/metabolism/).

# Science

## PERSPECTIVE

## On Getting There from Here

Steven L. McKnight

Studies on a variety of interesting biological problems, ranging from circadian rhythm to cancer cell growth to longevity, have begun to give evidence that the physiological state of cells and tissues reflects both the cell's regulatory systems and its state of intermediary metabolism. It is appreciated that the regulatory state of a cell or tissue, as driven by transcription factors and signaling pathways, can impose itself upon the dynamics of metabolic state. It follows that the reciprocal must also be the case, that metabolic state will feed back to impose itself on regulatory state. An appreciation and understanding of this reciprocity may be required to crack open problems in biological research that have heretofore been insoluble.

For the past 30 years, research in the biological sciences has been dominated by molecular biology. The successes of this approach have shaped our understanding of innumerable domains of biology. But any field that becomes sufficiently muscular can overshadow other credible approaches to scientific inquiry. One field etiolated by the cloud of molecular biology has been metabolism (Fig. 1). The vast majority of discoveries made by molecular biologists over the past several decades required no attention to the metabolic state of a cell. Molecular biologists needed no distracting thoughts about the metabolic state of a cell to discover microRNAs, the reprogramming of somatic cells into pluripotent stem cells, or gene rearrangement as the underlying basis for the generation of antibody diversity.

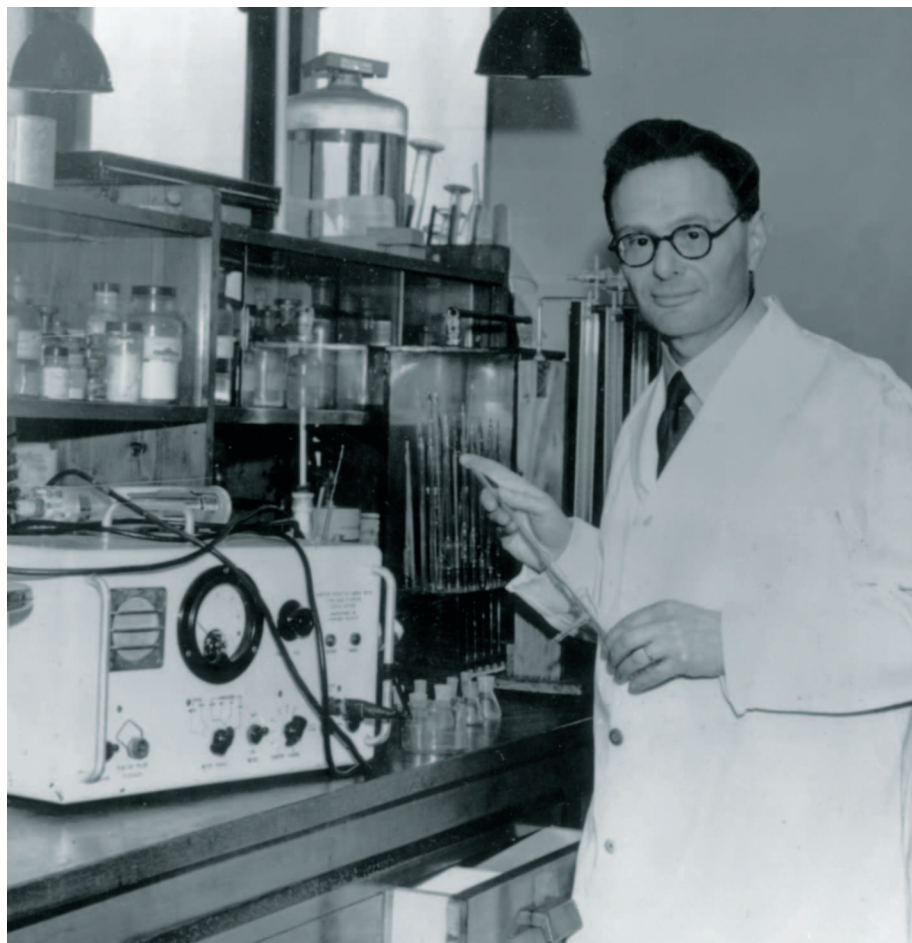
One simple way of looking at things is to consider that 9 questions out of 10 could be solved without thinking about metabolism at all, but the 10th question is simply intractable. As the saying goes, you simply "can't get there from here" to answer this 10th question if you are ignorant about the dynamics of metabolism. This, I propose, is where we are now finding pregnant opportunities in the field of experimental biology. The low-lying fruit that could be picked by molecular biologists without having to consider the metabolic state of a cell, tissue, or organism is largely gone. The more sticky problems that required attention to the dynamics of metabolism and that were pushed aside for decades now loom as interesting and important challenges.

Consider a prime example of how molecular biologists have begun to embrace the importance of metabolic regulation. Cancer researchers have long known of the enigmatic ability of tumor cells to undertake aerobic glycolysis, the so-called Warburg effect (1, 2). It makes sense that cancer cells would be highly glycolytic, yet why

would these cells choose to dispose of the terminal product of glycolysis? Instead of allowing pyruvate to be converted into acetyl-coenzyme A (CoA) via the spectacularly beautiful pyruvate dehydrogenase enzyme complex within mitochondria, cancer cells instead convert pyruvate into lactate through the lactate dehydrogenase enzyme, and then simply excrete it—blindly

giving away exceptional energetic value stored in the lactate hydrocarbon. Any cell that wants to grow—and there is nothing cancer cells care more about than growth—would be crazy to waste hydrocarbon; this would be akin to a motorist driving down the New Jersey Turnpike throwing away gasoline. The spendthrift waste of lactate likewise deprives the cancer cell of huge amounts of acetyl-CoA to be used for the synthesis of lipids, sterols, and other cellular building blocks. Despite progress, attention, and plenty of hype, it is safe to conclude that the famous Warburg effect remains a mystery.

Cancer researchers now recognize that regulatory proteins, such as the hypoxia-inducible transcription factors, can directly regulate the expression of genes encoding glycolytic enzymes (3). They now pay attention to how their favorite regulatory proteins, including the Myc and p53 transcription factors, help set the metabolic state of cells. The fact that these masterful transcription factors participate in dictating the metabolic state of a cell is now beginning to be accepted. The equally compelling corollary, however, remains largely unappreciated. That is, if regulatory state



**Fig. 1.** All eyes were focused on metabolism back in 1953 when Hans Krebs received the Nobel Prize for his discovery of the citric acid cycle.

Department of Biochemistry, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-9152, USA. E-mail: steven.mcknight@utsouthwestern.edu



(transcription factors, signaling pathways, etc.) is accepted to control metabolic state, is it not also unconditionally certain that metabolic state will reciprocally control the regulatory state itself? Understanding this reciprocity, and digging to the bottom of it, is where the future lies. Perhaps fittingly, this research will require the sophistication of scientists having genuine skills in the study of enzymology and intermediary metabolism.

Whole-genome sequencing efforts of individual tumors, now numbering in the thousands—yet soon to be numbered in the hundreds of thousands—are providing unbiased views of the myriad of “oncogenotypes” that underlie human cancer. Instead of ignoring mutations that happen to fall in the genes encoding metabolic enzymes, scientists seem more keen than ever to find and study such mutations. This change may reflect the recognition by cancer researchers that mutations that alter the function of metabolic enzymes might help to resolve the enigmatic, aerobic glycolytic state of certain cancer cells. It is equally likely that an understanding of how cancer cells veer away from normality with respect to intermediary metabolism might lead to the conceptualization of new and inventive strategies for therapeutic intervention.

For example, a set of recurrent genetic lesions believed to influence the metabolic state of glioblastoma cancer cells have been identified in the genes encoding either of the two isoforms of isocitrate dehydrogenase (4, 5). Perplexingly, most, if not all, of these lesions mutate a single arginine residue in either of the two isoforms of the enzyme (IDH1 or IDH2). The precise selectivity of the mutational events, coupled with the observation that only one allele of either enzyme appears to be mutated in human cancer, pointed to the possibility that the lesions might be causing the enzymes to adopt a new catalytic function. Indeed, the mutated forms of the IDH1 and IDH2 enzymes exhibit a reduced affinity for isocitrate and are endowed with a new catalytic function wherein  $\alpha$ -ketoglutarate is converted in an NADPH (nicotinamide adenine dinucleotide phosphate, reduced)–dependent manner to 2-hydroxyglutarate (6).

What pathways might be expected to be altered in a cell impeded for the production of  $\alpha$ -ketoglutarate and concomitantly endowed with increased intracellular production of 2-hydroxyglutarate? A logical guess would be the family of dioxygenase enzymes that use  $\alpha$ -ketoglutarate as an essential cofactor and, simultaneously, are inhibited in the presence of 2-hydroxyglutarate. Included among this family of dioxygenase enzymes are the prolyl-hydroxylase enzymes that modify

and negatively regulate the HIF-1 $\alpha$  (hypoxia-inducible factor 1 $\alpha$ ) and HIF-2 $\alpha$  transcription factors in oxygenated cells (7, 8). Partial elimination of these dioxygenase enzymes could be interpreted to lead to the activation of the hypoxia response pathway, thereby accounting for the activation of transcription of genes encoding glycolytic enzymes just as happens in the absence of the Von Hippel–Lindau (VHL) tumor suppressor gene (9, 10). This interpretation is very likely oversimplistic. Attenuation of  $\alpha$ -ketoglutarate concentrations and accumulation of increased amounts of 2-hydroxyglutarate almost certainly lead to the inhibition of other dioxygenase enzymes, of which mammalian cells have scores of isoforms. Intriguingly, some of these additional dioxygenase enzymes have been implicated in the control of histone demethylation (11–13), leaving open the possibility that changes in metabolic state might impose alterations in the epigenetic state of cancer cells.

The drift of this thinking is concordant with the recent discovery of mutations found in renal cell cancers in the mitochondrial enzyme fumarate hydratase that converts fumarate to malate (14). These mutations are more rare and of a recessive nature—wherein both alleles of the gene encoding fumarate hydratase must be inactivated. Because fumarate is known to be an inhibitor of the aforementioned family of dioxygenase enzymes, it is conceptually logical to hypothesize that the accumulation of excessive amounts of fumarate might inactivate the prolyl-hydroxylase enzymes that normally keep the HIF transcription factors in an inactive state (and, perhaps, likewise impose alterations in the epigenetic state of cancer cells). Finally, the same reasoning might apply to rare mutations in the human genes encoding mitochondrial succinate dehydrogenase and its assembly factors that are believed to contribute to the formation of paragangliomas and pheochromocytomas (15, 16). The exciting angle on these studies of cancer-causing mutations in the genes encoding isocitrate dehydrogenase, fumarate hydratase, and succinate dehydrogenase is that they formally predict the concept that the metabolic state of a cell can indeed exert control over its regulatory state, thereby confirming the reciprocal relationship between the two.

One enduring complication that shows little sign of resolution concerns the manner in which cancer cells are grown and studied in tissue culture plates. We routinely grow cancer cells under conditions of unlimited access to glucose—not to mention oxygen, vitamins, known and unknown growth factors present in serum, and every nutri-

tional fertilizer imaginable. The growth environment of cancer cells within tumors, especially solid tumors, could hardly be more different than that of cells being grown under standard, tissue culture conditions. It is reasonable to suspect that cancer cells weave their way through exceptionally selective oncogenetic gymnastics to achieve a growth-permissive metabolic state. If so, what features of this metabolic state can be anticipated to be preserved and studied when cells are practically grown in Karo syrup or alongside floating logs of Snickers Bar candy? Returning to the “you can’t get there from here” theme, it is predictable that we will have to find more biologically sound ways to grow cancer cells in culture in order to favorably use them in efforts to discover therapeutics that might exploit their unique metabolic state.

The resurrection of research involving or including metabolism is clearly upon us—that is the good news. The bad news is that the field was sufficiently snuffed over the past several decades that we have precious few scientists who have been trained to genuinely understand intermediary metabolism. Just because we can now pronounce the names of the metabolic enzymes whose encoding genes and mRNAs show up on our ChIP-Seq (chromatin immunoprecipitation–sequencing) and DNA microarrays lists does not necessarily mean that we can put two and two together. Despite the handicap of not being able to field an experienced team at this point, it is encouraging to see favorable trends that may enable negotiation of discovery routes that were, until now, largely obscure.

## References and Notes

1. O. Warburg, K. Posener, E. Negelein, *Biochem. Z.* **152**, 319 (1924).
2. O. Warburg, *Science* **123**, 309 (1956).
3. N. V. Iyer et al., *Genes Dev.* **12**, 149 (1998).
4. D. W. Parsons et al., *Science* **321**, 1807 (2008).
5. H. Yan et al., *N. Engl. J. Med.* **360**, 765 (2009).
6. L. Dang et al., *Nature* **462**, 739 (2009).
7. A. C. R. Epstein et al., *Cell* **107**, 43 (2001).
8. R. K. Bruick, S. L. McKnight, *Science* **294**, 1337 (2001).
9. P. H. Maxwell et al., *Nature* **399**, 271 (1999).
10. M. Ohh et al., *Nat. Cell Biol.* **2**, 423 (2000).
11. Y. Tsukada et al., *Nature* **439**, 811 (2006).
12. J. R. Whetstine et al., *Cell* **125**, 467 (2006).
13. P. A. Cloos et al., *Nature* **442**, 307 (2006).
14. J. S. Isaacs et al., *Cancer Cell* **8**, 143 (2005).
15. M. A. Selak et al., *Cancer Cell* **7**, 77 (2005).
16. H. X. Hao et al., *Science* **325**, 1139 (2009).
17. I thank M. Brown, R. Bruick, B. Tu, and J. Rutter for helpful editorial comments.

10.1126/science.1199908

# The Control of the Metabolic Switch in Cancers by Oncogenes and Tumor Suppressor Genes

Arnold J. Levine<sup>1,2\*</sup> and Anna M. Puzio-Kuter<sup>2</sup>

Cells from some tumors use an altered metabolic pattern compared with that of normal differentiated adult cells in the body. Tumor cells take up much more glucose and mainly process it through aerobic glycolysis, producing large quantities of secreted lactate with a lower use of oxidative phosphorylation that would generate more adenosine triphosphate (ATP), water, and carbon dioxide. This is the Warburg effect, which provides substrates for cell growth and division and free energy (ATP) from enhanced glucose use. This metabolic switch places the emphasis on producing intermediates for cell growth and division, and it is regulated by both oncogenes and tumor suppressor genes in a number of key cancer-producing pathways. Blocking these metabolic pathways or restoring these altered pathways could lead to a new approach in cancer treatments.

In 1926, Otto Warburg demonstrated that cancer cells did not metabolize glucose in the same way that glucose was catabolized in normal, adult differentiated cells (1, 2). The cancer cells relied on glycolysis, even in the presence of abundant oxygen (aerobic glycolysis) with a reduced use of the tricarboxylic acid (TCA) cycle, so that the pyruvate made in glycolysis was commonly converted to lactate, which was secreted from the cell (Fig. 1). Warburg suggested that this observation explained the cancer phenotype and was possibly a causal event in cancer formation (1, 2). There were several reasons why these observations were not understood and did not promote additional research. First, metabolizing glucose by glycolysis to produce pyruvate and secreted lactate is energetically inefficient. Most of the adenosine triphosphate (ATP) generated by glucose catabolism (34 out of 36 ATP molecules per molecule of glucose) occurs during the TCA cycle, which is used less often in cancer cells (Fig. 1). Second, it was unclear how this observation could contribute to the cancer phenotype. Third, possible mechanisms to mediate a switch to glucose utilization in glycolysis from the more efficient movement of pyruvate into the mitochondria to produce acetyl-coenzyme A (CoA) and enter the TCA cycle were only poorly understood. Fourth, with the discovery of the mutational activation of oncogenes and inactivation of tumor suppressor genes as causal steps in cancer, the relationship between these mutant genes and metabolic regulation was unclear. Remarkably, after a long absence of interest, research done in the past 10 years has

begun to answer these questions. Although our understanding of each question is still imperfect, it is becoming clear that both oncogenes and tumor suppressor gene products can influence the switch between aerobic glycolysis and a more extensive use of the TCA cycle to generate ATP. Furthermore, the altered metabolic processing of glucose observed by Warburg may well contribute to some of the causal changes in the cancer phenotype. There have been a number of reviews that emphasize different aspects of this question and provide a diverse set of answers (3–9).

Normal cells and cancer cells use both glucose and glutamine as substrates to generate energy for the cell (ATP); to produce substrates to synthesize amino acids, nucleosides, and fatty acids; and to regulate the redox potential (number of oxidized molecules in a compartment divided by number of reduced molecules) so as to minimize the effects of reactive oxygen species (ROS) that damage membranes and proteins and cause mutations in a cell. Glucose contributes carbon, oxygen, and hydrogen for both anabolic processes and energy, whereas glutamine contributes nitrogen for synthesis of purines, pyrimidines, and nonessential amino acids. Metabolism of glutamine also produces the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) for the synthesis of fatty acids and the modulation of the redox potential in a cell. Glucose passing through the pentose phosphate pathway (PPP) also generates NADPH and ribose-5-phosphate for the synthesis of nucleotides (Fig. 1). Normal adult differentiated cells have a low cell division rate (low turnover) and predominantly metabolize glucose to CO<sub>2</sub> and H<sub>2</sub>O through glycolysis and the TCA cycle. This satisfies the needs of these cells for free energy supplied by efficient ATP generation during oxidative phosphorylation (complexes 1 to 4 in the oxidative phosphorylation chain) linked to the TCA cycle in mitochondria.

There are several times, however, when regulated rapid cell division is required, such as during embryonic development, in wound healing (liver regeneration), or in the immune responses to specific antigens, where clonal selection provides increased cell numbers with increased immune specificity. Cancer cells share many of these same requirements for energy, substrates to grow and divide, and control of the redox potential and ROS in the cell.

What these processes have in common is a need to synthesize substrates for membranes, nucleic acids, and proteins (increase mass), which means not metabolizing all of the glucose to CO<sub>2</sub> and H<sub>2</sub>O but instead providing the proper intermediates for cell growth. This is accomplished, in part, by slowing the entry of pyruvate into mitochondria, decreasing the conversion to acetyl-CoA, and slowing the rate of the TCA cycle. The pyruvate that builds up in aerobic glycolysis is, in part, converted into lactate that is secreted, eliminating it from the pool and keeping glycolysis active. The secreted lactate lowers the pH of the cellular environment and the extracellular matrix. This may influence remodeling of the matrix, permitting blood vessel invasion in response to angiogenic factors produced by the tumor (10). Furthermore, as a consequence of glycolysis, tumor lesions can become acidotic, which allows for the selection of motile cells that can break through the basement membrane and metastasize. The last step in glycolysis is catalyzed by pyruvate kinase, which receives input about both anabolic precursors and the energy status of the cell. Cancer cells make the fetal isoform of pyruvate kinase (the M2 isoform), which is a spliced variant of the gene that adds several amino acids, one of which is a tyrosine. This tyrosine is phosphorylated in cells with activated tyrosine kinase signaling, a hallmark of actively growing cells. Pyruvate kinase M2 is stimulated in a feedforward loop by fructose 1,6-bisphosphate, but the phosphotyrosine inhibits this positive regulation. Thus, in cancer cells the last step of glycolysis is slowed, resulting in a buildup of phosphorylated intermediates that can be used in anabolic synthesis and cell growth (11).

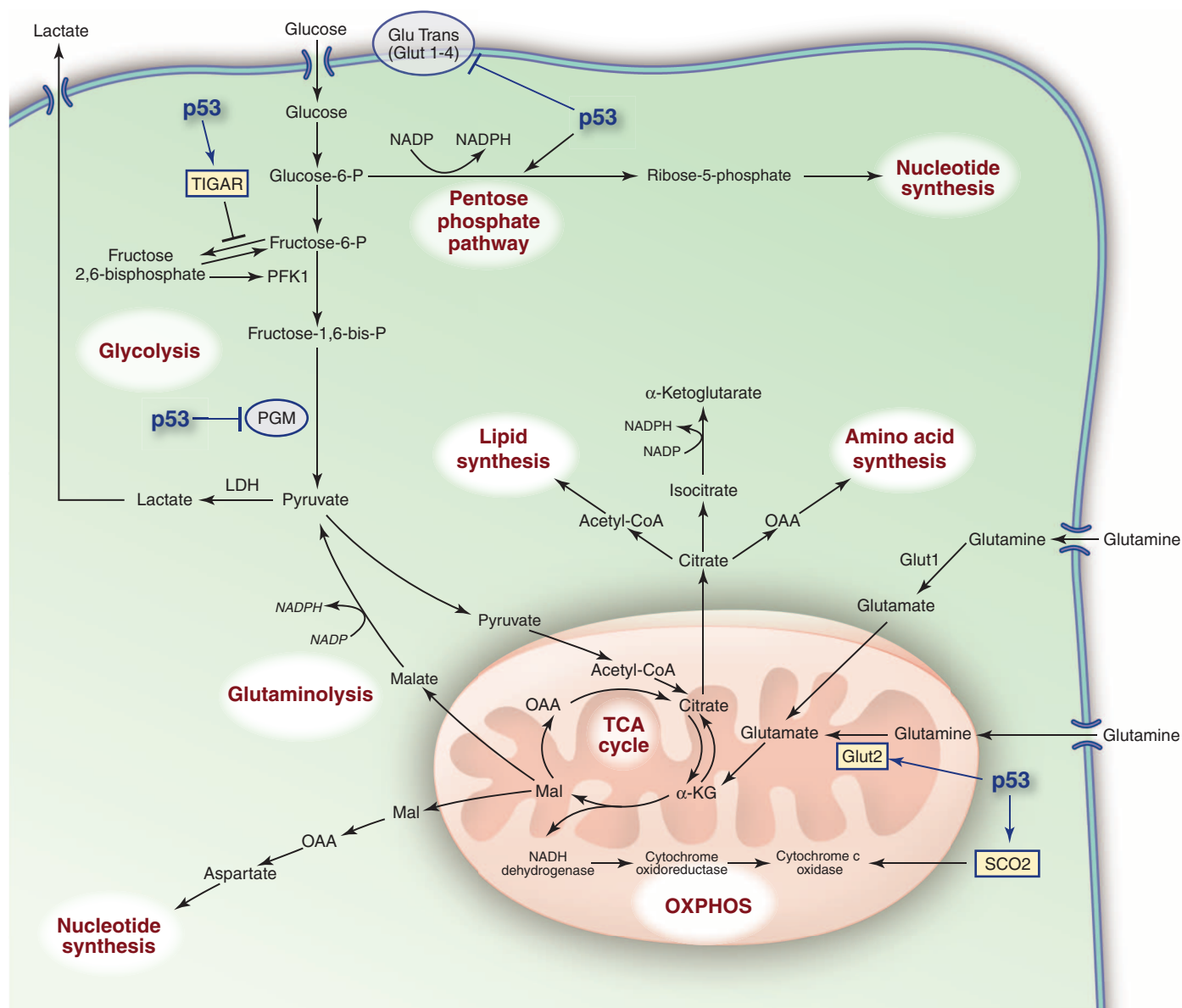
Rapidly dividing cells require favorable energetics [that is, higher ATP/adenosine diphosphate (ADP) and ATP/adenosine monophosphate (AMP) ratios]. Many cancer cells satisfy this problem by taking up much larger amounts of glucose than do normal cells. This results from facilitated glucose transport by one or more of several isozymes of membrane glucose transporters (GLUT 1 to 9). Once inside the cell, glucose is phosphorylated by one of several hexokinase enzymes (the first step in glycolysis) to keep it in the cell because of the charge added to glucose (Fig. 1). The high concentrations of glucose in the cells of a cancer may be observed by positron emission tomography (PET) scans of radioactive F-19-2-deoxyglucose (FDG is not metabolized but is located in the

<sup>1</sup>Institute for Advanced Study, Princeton, NJ 08540, USA.

<sup>2</sup>Cancer Institute of New Jersey, New Brunswick, NJ 08903, USA.

\*To whom correspondence should be addressed. E-mail: alevine@ias.edu





**Fig. 1.** Signaling networks and their regulation of metabolism in proliferating cells. The figure shows aspects of metabolism in proliferating cells including glycolysis; lactate production; TCA cycle; oxidative phosphorylation; PPP; glutaminolysis; and the biosynthesis of nucleotides, lipids, and amino acids. Glucose can be processed through glycolysis for production of ATP and pyruvate, pass through the PPP to generate ribose 5-phosphate and NADPH, and also enter into the mitochondrion-localized TCA cycle. Glucose-derived citrate is exported to the cytosol and processed to acetyl-CoA, oxaloacetate (OAA), or  $\alpha$ -ketoglutarate ( $\alpha$ -KG). Glutamine

is deaminated to form glutamate, which is processed to generate  $\alpha$ -ketoglutarate and maintain the TCA cycle. p53 induction of key players is boxed, and p53 inhibition is circled. p53 induces TIGAR, inhibits phosphoglycerate mutase (PGM), and represses GLUT1 and GLUT 4, resulting in inhibition of glycolysis and opposing the Warburg effect that is seen in many cancers, whereas p53 induction of SCO2 and GLS2 enhances mitochondrial respiration. Glut Trans indicates glucose transporters; Glut 1, glutaminase 1; Glut 2, glutaminase 2; LDH, lactate dehydrogenase; Mal, malate; and OXPHOS, oxidative phosphorylation.

cell), which is indicative of enhanced glucose uptake by cells. Many, but not all, cancers have this property (3–9) of increasing glucose uptake, and this is a confirmation of the Warburg effect.

With large amounts of glucose available in a cell, glucose is metabolized through the PPP, producing nucleosides and generating NADPH. The NADPH is essential for fatty acid synthesis, along with acetyl-CoA (which is made from some

of the pyruvate in mitochondria that is not converted to lactate). NADPH also contributes to a proper redox control and protects the cell from ROS. There are several ways the cell responds to lower ROS levels, but by far the major molecule involved is glutathione (GSH), which eliminates ROS by accepting an electron and is converted to its oxidized form, GSSG (glutathione disulfide). The enzyme glutathione reductase uses NADPH

to reduce GSSG to GSH. Thus, NADPH is a major source of cellular “coolant” when oxidative reactions run too “hot” (high ROS levels) by using large amounts of glucose to produce both substrates and energy. However, high levels of ROS can be advantageous for cancer cells when they allow for the stimulation of cell proliferation, induction of genetic instability, and evasion from senescence. Although if levels are too high, then

cancer cells undergo oxidative damage-induced cell death. Thus, ROS levels can be exploited to selectively kill cancer cells and therefore be used as a potential therapeutic.

Glutamine contributes both to substrate needs of a dividing cell and to control of redox potentials through the synthesis of NADPH. As with glucose, excessive amounts of glutamine are taken up (by a glutamine transporter) and used by cancer cells. After glutamine is taken into the cell, a mitochondrial-associated enzyme, glutaminase-1, converts it to glutamate (a transaminase can use the amino group and capture the nitrogen for synthesis of nucleosides, and amino acids or ammonium is produced). Glutamate is converted to  $\alpha$ -ketoglutarate and enters the TCA cycle in the mitochondria. The malate and citrate produced in the TCA cycle leave the mitochondria, where malate is converted to pyruvate plus NADPH and citrate is converted to isocitrate and then to  $\alpha$ -ketoglutarate, generating another molecule of NADPH. Citrate also is converted to acetyl-CoA for fatty acid synthesis and oxaloacetate for the synthesis of nonessential amino acids (Fig. 1). The pyruvate generated in these reactions can be used to produce glucose (reverse glycolysis), which enters the PPP, maximizing the production of NADPH. The glutamate can be converted to aspartate, which contributes to nucleoside synthesis. The excessive quantities of glutamine taken into and used by the cell results in the secretion of alanine and ammonium, which together with lactate bathe the extracellular matrix.

Glutamine is also a major cancer cell energy and anabolic substrate that requires functional mitochondria. However, Warburg's hypothesis had its basis in the theory that glycolysis is predominately used in cancer cells because of a dysregulation of mitochondrial oxidative phosphorylation. Research has shown that most cancer cells do not have defects in mitochondrial metabolism except for rare mutations in succinate dehydrogenase (SDH) or fumarate hydratase (FH), both enzymes of the TCA cycle and both initiating events of familial paraganglioma or leiomyoma and of papillary renal cell cancer.

Thus, cancer cells maximize their ability to synthesize substrates for membranes, nucleic acids, and proteins. This results in increased cell mass and allows cell division when needed. This cannot be accomplished without large amounts of energy (ATP), which are obtained by increasing the use of glucose and glutamine many fold. Penalties for this increased flux are an increase in oxidative intermediates, an altered redox potential, and excessive ROS. The metabolic response is to focus reactions on producing NADPH, a coolant that feeds into many chemical systems that reduce the ROS activity. This high rate of glucose and glutamine flux must be handled by increased metabolic enzyme levels or increased enzyme activities. Remarkably, this is accomplished by oncogenes and tumor suppressor genes

as well as regulators of the response to hypoxia. All of these metabolic pathways (TCA, PPP, and glycolysis) contain complex regulatory circuits at the levels of transcription, mRNA splicing, translation, and small molecule feedforward and feedback loops. A deeper understanding of these regulatory pathways that connect the genetics of cancer to the biochemical metabolic pathways may reveal selected metabolic processes that might be good drug targets for slowing or reversing cancers.

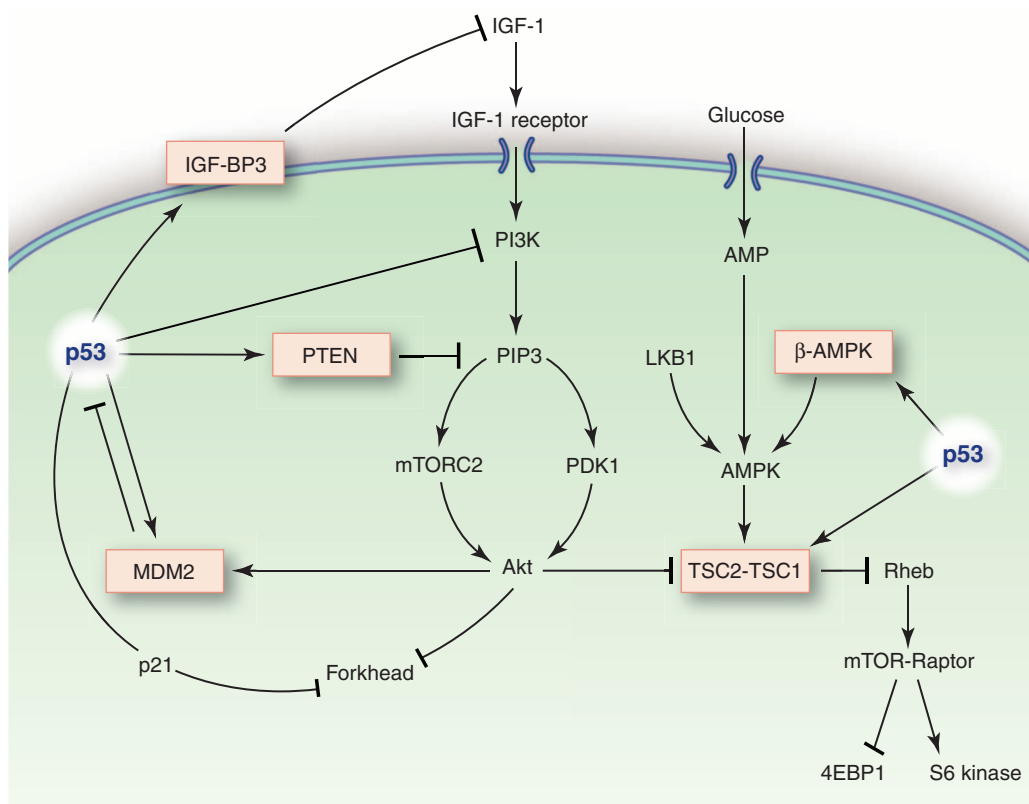
Over the past 10 years, evidence has accumulated that the oncogenes *myc*, nuclear factor  $\kappa$ B (NF- $\kappa$ B), AKT, and the tyrosine kinase receptors (epidermal growth factor, EGF; insulin-like growth factor 1, IGF-1; Her-2; etc.), which turn on Ras, RAF-mitogen-activated protein kinase (MAP kinase), and the phosphatidylinositol 3-kinases (PI3Ks) and mammalian target of rapamycin (mTOR) pathways (Fig. 2) along with hypoxia-induced factor (HIF), can stimulate the transcription of a number of genes that encode the proteins that mediate the glycolysis and glutaminolysis pathways (Fig. 1). The rate of glycolysis can vary over 100-fold. High AKT and mTOR activities result in high HIF activity. Both the *myc* and HIF-1 transcription factors increase the rate of transcription of some of the GLUT transporters and hexokinase 2, enhancing both glucose uptake and its retention in the cell (11). HIF increases the rate of transcription of over 100 genes, resulting in angiogenesis, cell migration, cell survival, and energy metabolism.

Among the HIF-regulated genes are 9 of the 10 enzymes that function in glycolysis (12, 13). HIF is regulated by the cellular hypoxia response. Acute hypoxia stabilizes the HIF-1 $\alpha$  and HIF-2 $\alpha$  proteins, which form dimers with HIF-1 $\beta$  and HIF-2 $\beta$ . The stability of HIF $\alpha$  subunits is controlled by HIF prolyl-hydroxylases (PHDs), which use O<sub>2</sub> and  $\alpha$ -ketoglutarate to convert a prolyl residue to hydroxyproline plus succinate and CO<sub>2</sub> (14, 15). The hydroxyl-proline residues are bound by the von Hippel-Lindau protein complex (VHL tumor suppressor lost in some types of cancers) containing an E3 ubiquitin ligase, which results in the HIF $\alpha$  subunit being polyubiquitinated and degraded (16). The absence of oxygen stabilizes the HIF transcription factor. In addition, lactate dehydrogenase A and pyruvate dehydrogenase kinase are transcriptionally regulated by HIF, both of which keep pyruvate away from the mitochondria. The loss of PTEN (phosphatase and tensin homolog, a tumor suppressor gene) and concurrent increase of AKT-1 and mTOR lead to HIF activation and the Warburg effect (Fig. 2). The *myc* transcription factor activates the transcription of more than 1000 genes involved in all phases of cell growth and metabolism. *Myc* enhances the transcription of glutaminase-1, the first enzyme in glutaminolysis producing glutamate (17), and it transcribes the ribosomal RNA genes and the ribosomal protein genes, increasing the rate of

protein synthesis and mass of a cell (18). *Myc* also regulates glutaminolysis at the microRNA (miRNA) level by transcriptionally repressing miR-23a and miR-23b, which results in greater expression of their target protein, mitochondrial glutaminase-1, and thus up-regulation of glutamine catabolism.

Similarly, the loss of p53 functions lead to the Warburg effect (Fig. 2). The p53 protein represses the transcription of the GLUT 1 and 4 transporters (18). The p53 protein induces the transcription of the TIGAR gene, which lowers the intracellular concentrations of fructose 2,6 biphosphatase (FBPase) and thus decreases glycolysis by diverting glucose through the PPP (19) (Fig. 1). TIGAR also has functional similarities to the biphosphate domain of PFK-2/FBPase-2 in regulating glycolysis, ROS levels, and apoptosis and is structurally similar to FBPase2. The activation of p53 also increases the ubiquitination of phosphoglycerol mutase, which decreases the activity of this glycolytic enzyme. p53 increases the use of the TCA cycle and oxidative phosphorylation. The p53 protein enhances the transcription of the gene for synthesis of cytochrome c oxidase 2 (SCO2), which, along with synthesis of cytochrome c oxidase 1 (SCO1), assembles into oxidative phosphorylation complexes (20). Cells with mutant p53 have compromised oxidative phosphorylation chains. p53 also promotes synthesis of a number of proteins that reduce the high ROS load in cells. Sestrins 1 to 4 are p53-regulated genes and produce proteins that react with and neutralize ROS (21). p53 also regulates the p21 gene, and the p21 protein binds to and stabilizes the Nrf2 transcription factor, which regulates a set of complex responses to altered redox potentials and high ROS. P53 transcribes the glutaminase 2 gene, a nuclear gene that produces a glutaminase localized in the internal compartment of mitochondria (22, 23). Unlike glutaminase 1, glutaminase 2 converts glutamine to glutamate, which can be used to enhance the rate of the TCA cycle and oxidative phosphorylation (22, 23). Thus, these two glutaminases, regulated by *myc* (glutaminase 1) and p53 (glutaminase 2), have opposite effects on the cell. Just why this is the case remains to be elucidated. An activated p53 protein also inhibits the activities of the phosphatidylinositol-3 kinase (PI3K)-AKT and mTOR pathways (Fig. 2). P53 regulates the transcription of four genes, PTEN, IGF-binding protein-3 (IGF-1BP-3), tuberous sclerosis protein 2 (TSC-2), and the beta subunit of AMP-activated protein kinase (AMPK), which all negatively regulate AKT kinase and mTOR (24, 25). In addition, sestrins 1 and 2, which are p53-regulated genes, stimulate AMPK activity (26). All of these activities shut down cell growth, decrease the Warburg effect, lower HIF levels, and thus reverse the cancer phenotype. In some cases, this results in a p53-directed apoptosis and the activation of autophagy (27).





**Fig. 2.** p53 regulation of PI3K, Akt, and mTOR pathways. p53 functions in a complex network to mediate a cell's adaptation to stress. To do this, p53 regulates the transcription of four genes, PTEN, IGF-BP3, TSC2, and AMPK $\beta$ , which then all negatively regulate Akt kinase and mTOR, leading to a decrease in cell growth and a reversal of the cancer phenotype. Coordinately, there is an inhibition of proliferation that is promoted through an activation of p53 and LKB1. In addition to slowing cell growth, p53-dependent inhibition of the mTOR pathway promotes autophagy, a way of helping cells survive. 4EBP1, 4E-binding protein 1; IGF-1 receptor, insulin-like growth factor-1 receptor; LKB1, liver kinase B1; MDM2, murine double minute 2; PDK1, phosphoinositide-dependent kinase-1; PIP3, phosphatidylinositol 3,4,5-trisphosphate; Raptor, regulatory associated protein of mTOR; Rheb, Ras homolog enriched in brain; S6 kinase, ribosomal protein S6 kinase; TSC, tuberous sclerosis complex.

A number of mutations in genes that encode enzymes in the TCA cycle have been shown to lead to some types of cancers. Mutations in succinate dehydrogenase and fumarate hydratase alter the complex 2 oxidative phosphorylation chain, which generates reduced flavine adenine dinucleotide (FADH<sub>2</sub>). These mutations force a switch to the Warburg effect and contribute to selected inherited and sporadic cancers (27). There is some evidence that these mutations result in the inactivation of the PHDs, leading to increases in HIF-1 and an enhanced glycolytic pathway. In glioblastoma multiforme, up to 12% of these tumors have spontaneous point mutations in the gene for cytosolic isocitrate dehydrogenase 1 (IDH1) (28). This enzyme converts isocitrate to  $\alpha$ -ketoglutarate, generating NADPH. Likewise, mutations have been observed in IDH2 at residue Arg<sup>172</sup>, in the active site, in patients of low-grade gliomas (29) and acute myeloid leukemia (AML) (30), as well as other diseases (31). The somatic mutations in IDH1 and IDH2 identified in gliomas and AML result in a new ability of the enzyme to catalyze the

NADH-dependent reduction of  $\alpha$ -ketoglutarate to 2-hydroxyglutarate, an oncometabolite that can be a correlative marker for mutations occurring in isocitrate dehydrogenase enzymes (32). Just why this is so strongly selected for in these tumors may well be more complex than simply generating more NADPH.

The large number of genetic alterations observed in human cancers in the oncogenes and tumor suppressor genes involved in the IGF-1/mTOR pathways (Fig. 2) suggest that drugs may be developed that alter the Warburg effect and some of its consequences. Inhibitors of TOR complex1 (TORC1), which controls protein synthesis and cell cycle progression, are already approved for use in selected cancers. TORC1 is regulated by AMPK, which measures ATP/AMP ratios and nutrient availability. Metformin, which is used as a treatment for type 2 diabetes, stimulates AMPK. Diabetic patients treated with metformin have lower incidences of cancer than diabetics not treated with this drug (33, 34). Indeed, metformin acts as a synthetic lethal drug on cells in culture

that contain p53 mutations, demonstrating the close interactions between these two pathways (35). The inhibition of lactate dehydrogenase in cancer cells slows their growth, suggesting the importance of making and secreting lactate from cancer cells. Most hepatocellular carcinomas have lost the expression of glutaminase-2 in mitochondria, even though most of these tumors do not contain p53 mutations (p53 regulates this gene). Returning a cDNA for glutaminase-2 to these cells in culture and expressing this protein, which enhances the use of the TCA cycle, inhibits cell division (22, 23). Thus, glutaminase-2 is acting like a tumor suppressor gene in these situations. Indeed, a wild-type p53 gene and protein are required for efficient mitochondrial DNA replication and mitochondrial maintenance in cells (36). These types of observations suggest that the extensive alterations of metabolic processes in cancer can contribute to the phenotypes of the tumor cells and as such are themselves causal (necessary but not sufficient) for these cancers.

## Conclusions

The observations and ideas reviewed here suggest a unity in the genes and pathways involved in several diseases. The interrelationships of the p53, AKT, and mTOR pathways (Fig. 2) bring together stress responses and diabetes. Indeed, p53 in adipose tissue can regulate insulin resistance (37). There is a similar overlap among several genes whose mutations predispose an individual to Parkinson's disease and the functions of those genes in the p53, AKT, and mTOR pathways (38). The connections among chronic inflammatory responses of the immune system, with the activation of NF- $\kappa$ B and its associated metabolic changes (Warburg effect) and PET scan-positive cells, and the formation of cancers of those cells are well established (39). It should not be surprising to observe such a central role of metabolic processes in many disorders and the integration of metabolic pathways with many diverse signal transduction pathways. Metabolic pathways comprise an evolutionarily conserved underlying feature for most functions of a cell and an organism.

## References

1. O. Warburg, K. Posener, E. Negelein, *Biochem. Z.* **152**, 309 (1924).
2. O. Warburg, *Science* **123**, 309 (1956).
3. P. P. Hsu, D. M. Sabatini, *Cell* **134**, 703 (2008).
4. G. Kroemer, J. Pouyssegur, *Cancer Cell* **13**, 472 (2008).

5. M. G. Vander Heiden, L. C. Cantley, C. B. Thompson, *Science* **324**, 1029 (2009).
6. R. J. DeBerardinis, N. Sayed, D. Ditsworth, C. B. Thompson, *Curr. Opin. Genet. Dev.* **18**, 54 (2008).
7. J. W. Locasale, L. C. Cantley, M. G. Vander Heiden, *Nat. Biotechnol.* **27**, 916 (2009).
8. D. A. Tennant, R. V. Durán, H. Boulahbel, E. Gottlieb, *Carcinogenesis* **30**, 1269 (2009).
9. E. Gottlieb, K. H. Vousden, in *The p53 Family*, A. J. Levine, D. Lane, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2010), pp. 187–198.
10. T. K. Hunt *et al.*, *Antioxid. Redox Signal.* **9**, 1115 (2007).
11. H. R. Christofk, M. G. Vander Heiden, N. Wu, J. M. Asara, L. C. Cantley, *Nature* **452**, 181 (2008).
12. G. L. Semenza, *Nat. Rev. Cancer* **3**, 721 (2003).
13. M. L. Macheda, S. Rogers, J. D. Best, *J. Cell. Physiol.* **202**, 654 (2005).
14. R. K. Bruick, S. L. McKnight, *Science* **294**, 1337 (2001); 10.1126/science.1066373.
15. K. S. Hewitson, L. A. McNeill, J. M. Elkins, C. J. Schofield, *Biochem. Soc. Trans.* **31**, 510 (2003).
16. P. H. Maxwell *et al.*, *Nature* **399**, 271 (1999).
17. R. J. DeBerardinis *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 19345 (2007).
18. C. V. Dang, *Mol. Cell. Biol.* **19**, 1 (1999).
19. K. Bensaad *et al.*, *Cell* **126**, 107 (2006).
20. S. Matoba *et al.*, *Science* **312**, 1650 (2006); 10.1126/science.1126863.
21. A. V. Budanov, A. A. Sablina, E. Feinstein, E. V. Koonin, P. M. Chumakov, *Science* **304**, 596 (2004).
22. W. Hu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 7455 (2010).
23. S. Suzuki *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 7461 (2010).
24. Z. Feng *et al.*, *Cancer Res.* **67**, 3043 (2007).
25. Z. Feng, in *The p53 Family*, A. J. Levine, D. Lane, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2010), pp. 199–298.
26. A. V. Budanov, M. Karin, *Cell* **134**, 451 (2008).
27. Z. Feng, H. Zhang, A. J. Levine, S. Jin, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 8204 (2005).
28. D. W. Parsons *et al.*, *Science* **321**, 1807 (2008); 10.1126/science.1164382.
29. H. Yan *et al.*, *N. Engl. J. Med.* **19**, 765 (2009).
30. P. S. Ward *et al.*, *Cancer Cell* **16**, 225 (2010).
31. M. Kranendijk *et al.*, *Science* **330**, 336 (2010); 10.1126/science.1192632.
32. L. Dang *et al.*, *Nature* **17**, 966 (2010).
33. J. M. Evans, L. A. Donnelly, A. M. Emslie-Smith, D. R. Alessi, A. D. Morris, *BMJ* **330**, 1304 (2005).
34. S. Jiralerspong *et al.*, *J. Clin. Oncol.* **27**, 3297 (2009).
35. M. Buzzai *et al.*, *Cancer Res.* **67**, 6745 (2007).
36. A. Bourdon *et al.*, *Nat. Genet.* **39**, 776 (2007).
37. T. Minamino *et al.*, *Nat. Med.* **15**, 1082 (2009).
38. R. H. Kim *et al.*, *Cancer Cell* **7**, 263 (2005).
39. P. Ak, A. J. Levine, *FASEB J.* **24**, 3643 (2010).

10.1126/science.1193494

## REVIEW

# Autophagy and Metabolism

Joshua D. Rabinowitz<sup>1,2</sup> and Eileen White<sup>2,3,4</sup>

Autophagy is a process of self-cannibalization. Cells capture their own cytoplasm and organelles and consume them in lysosomes. The resulting breakdown products are inputs to cellular metabolism, through which they are used to generate energy and to build new proteins and membranes. Autophagy preserves the health of cells and tissues by replacing outdated and damaged cellular components with fresh ones. In starvation, it provides an internal source of nutrients for energy generation and, thus, survival. A powerful promoter of metabolic homeostasis at both the cellular and whole-animal level, autophagy prevents degenerative diseases. It does have a downside, however—cancer cells exploit it to survive in nutrient-poor tumors.

Living organisms from yeast to humans are capable of eating parts of themselves in order to survive. This involves the degradation of cellular components, either because they are deleterious (e.g., damaged organelles and microbial invaders) or because the resulting breakdown products are needed to support metabolism. This process was aptly termed autophagy from the Greek “auto” or oneself and “phagy” or to eat. It has gained attention recently as an essential contributor to human health and disease.

There are several forms of autophagy, each of which involves delivering intracellular cargo to lysosomes for degradation. The predominant form, macroautophagy (autophagy hereafter), produces vesicles called autophagosomes that capture and deliver cytoplasmic material to lysosomes (*1*). The autophagy-related genes (the *atg* genes) are

conserved from yeast to mammals and regulate the cannibalism of intracellular cytoplasm, proteins, and organelles.

Autophagy is the only mechanism to degrade large structures such as organelles and protein aggregates. In the absence of stress, basal autophagy serves a housekeeping function. It provides a routine “garbage disposal” service to cells, eliminating damaged components that could otherwise become toxic. Such cellular refreshing is particularly important in quiescent and terminally differentiated cells, where damaged components are not diluted by cell replication. In starvation, autophagy provides a nutrient source, promoting survival. Autophagy is induced by a broad range of other stressors and can degrade protein aggregates, oxidized lipids, damaged organelles, and even intracellular pathogens. Although it is not always possible to resolve the metabolic and garbage disposal roles for autophagy, it is clear that autophagy prevents disease. Defects in autophagy are linked to liver disease, neurodegeneration, Crohn’s disease, aging, cancer, and metabolic syndrome.

## Process of Autophagy

A series of protein complexes composed of *atg* gene products coordinate the formation of auto-

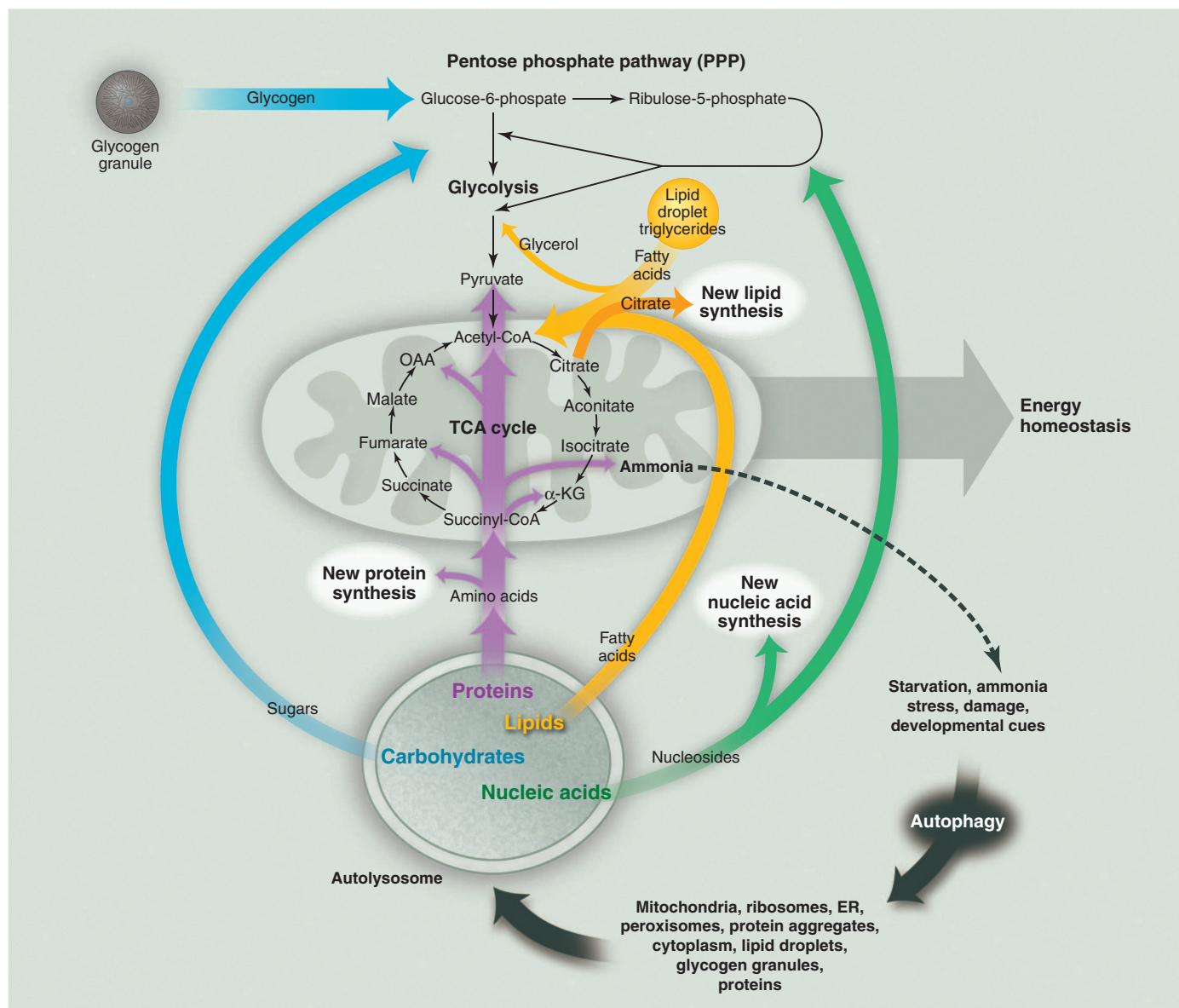
phagosomes. The Atg1/ULK1 complex (Atg1 in yeast and ULK1 in mammals) is an essential positive regulator of autophagosome formation (*1*). When nutrients are abundant, binding of the ULK1 complex by the mammalian target of rapamycin (mTOR) complex 1 (mTORC1) inhibits autophagy. mTORC1 is an important regulator of cell growth and metabolism. It is composed of five subunits that include Raptor, which binds ULK1, and mTOR, a serine-threonine kinase. By phosphorylating ULK1 and another complex member (the mammalian homolog of yeast Atg13), mTOR inhibits autophagy initiation. In starvation, mTORC1 dissociates from the ULK1 complex, freeing it to trigger autophagosome nucleation and elongation.

Autophagosome nucleation requires a complex containing Atg6 or its mammalian homolog, Beclin 1, that recruits the class III phosphatidylinositol 3-kinase VPS34 to generate phosphatidylinositol 3-phosphate (*2*). Expansion of autophagosome membranes involves two ubiquitin-like molecules, Atg12 and Atg8 (called LC3 in mammals), and two associated conjugation systems. The E1-like Atg7 and E2-like Atg10 covalently link Atg12 with Atg5, which together bind Atg16L1 to form pre-autophagosomal structures. In the second ubiquitin-like reaction, LC3 is cleaved by the protease Atg4. Phosphatidylethanolamine is conjugated to cleaved LC3 by Atg7 and a second E2-like enzyme, Atg3, and this lipidated LC3-II associates with newly forming autophagosome membranes. LC3-II remains on mature autophagosomes until after fusion with lysosomes and is commonly used to monitor autophagy.

The process beginning with the Beclin 1 complex gives rise to nascent autophagosome membranes. These membranes assemble around cargo, encapsulating the cargo in a vesicle that subsequently fuses with a lysosome, generating an autolysosome. The contents are then degraded by proteases, lipases, nucleases, and glycosidases. Lysosomal permeases release the breakdown products—amino acids, lipids, nucleosides, and carbohydrates—into the cytosol, where they are

<sup>1</sup>Department of Chemistry and Lewis-Sigler Institute for Integrative Genomics, 241 Carl Icahn Laboratory, Washington Road, Princeton University, Princeton, NJ 08544, USA. E-mail: joshjr@genomics.princeton.edu <sup>2</sup>Cancer Institute of New Jersey, 195 Little Albany Street, New Brunswick, NJ 08903, USA. <sup>3</sup>Department of Molecular Biology and Biochemistry, Rutgers University, 604 Allison Road, Piscataway, NJ 08854, USA. <sup>4</sup>Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 675 Hoes Lane, Piscataway, NJ 08854, USA. E-mail: whiteei@umdnj.edu





**Fig. 1.** Use of the products of autophagy. Multiple forms of stress activate autophagy (bottom right). Degradation of proteins, lipids, carbohydrates, and nucleic acids liberates amino acids, fatty acids, sugars, and nucleosides that are released into the cytoplasm for reutilization. Sugars (blue lines), including glucose released from glycogen granules by glycogenolysis or autophagy, are catabolized by glycolysis and the PPP to generate ATP, and pyruvate for subsequent TCA cycle metabolism. Nucleosides (green lines) are used for new nucleic acid synthesis and catabolized by the combined action of the PPP and glycolysis. Amino acids (purple

lines) are used as building blocks for new protein synthesis, for ATP production by central carbon metabolism, and (in liver) as substrates for gluconeogenesis (Fig. 3). They also can be combined to yield citrate, which drives lipid synthesis and membrane biogenesis. Catabolism of amino acids yields ammonia, an activator of autophagy (dotted line). Fatty acids (yellow lines) from lipolysis or from autophagy of membranes or lipid droplets yield acetyl-CoA, which feeds the TCA cycle, supporting ATP production and citrate generation. OAA indicates oxaloacetate;  $\alpha$ -KG,  $\alpha$ -ketoglutarate; and ER, endoplasmic reticulum.

available for synthetic and metabolic pathways (Fig. 1).

### Substrates of Autophagy

Autophagy can be nonselective or selective. Non-selective, bulk degradation of cytoplasm and organelles by autophagy provides material to support metabolism during starvation. It also contributes to extensive tissue remodeling, as in *Drosophila* morphogenesis (3). Whether mechanisms exist to

prevent bulk autophagy from consuming essential components, such as a cell's final mitochondrion, remains unclear, and in some cases such consumption may lead to cell death.

Selective autophagy of proteins and of organelles such as mitochondria (mitophagy), ribosomes (ribophagy), endoplasmic reticulum (reticulophagy), peroxisomes (pexophagy), and lipids (lipophagy) occurs in specific situations. In mammals, the signal for targeting proteins for

degradation by either the autophagy or proteasome pathways is ubiquitination. Many proteins accumulate in autophagy-defective mammalian cells, indicating that autophagy has a major role in controlling the cellular proteome and that proteasome-mediated degradation cannot compensate for defective autophagy (4). To target proteins for autophagic degradation, ubiquitin on modified proteins is recognized and bound by autophagy receptors, such as p62 or Nbr1, which

interact with LC3 to deliver cargo to autophagosomes (1).

Mechanisms regulating selective autophagy of organelles are more elaborate. In yeast, Atg32 localized in mitochondria is a receptor that interacts with Atg8 and Atg11 to produce selective mitochondrial autophagy (5, 6). In mammals, autophagy of depolarized mitochondria, which protects cells from toxic reactive oxygen species, is initiated by Pink1-dependent mitochondrial translocation of Parkin. This is followed by ubiquitination of mitochondrial proteins and recruitment of p62 to direct mitochondria to autophagosomes (7, 8). The pruning of damaged mitochondria by autophagy has two homeostatic functions. The first is limiting oxidative damage. The second is in maintaining a functional mitochondrial pool.

## Regulation of Autophagy

Cells integrate information regarding nutrient availability, growth factor and hormonal receptor activation, stress, and internal energy through an elaborate array of signaling pathways (Fig. 2). In mammals, insulin—the master hormone of the fed state—blocks autophagy.

A major intracellular hub for integrating autophagy-related signals is mTORC1 (9). In the presence of abundant nutrients and growth factors including insulin, mTORC1 promotes cell growth and metabolic activity while suppressing the ULK1 complex and autophagy. In deprivation or stress, numerous signaling pathways inactivate mTORC1 kinase activity. This both suppresses cell growth to reduce energy demand and induces autophagy to enable stress adaptation and survival. A second mTOR complex, mTORC2, positively regulates mTORC1. Upstream of mTORC1 is the cellular energy-sensing pathway controlled by adenosine monophosphate-activated protein kinase (AMPK) (10). High concentrations of AMP signal energy depletion, activate AMPK, and inhibit mTORC1, thus promoting autophagy (Fig. 2).

Regulation of autophagy also occurs through the forkhead box or FOXO transcription factors, whose activation leads to transcription of *atg* genes (11). Similarly, hypoxia and activation of hypoxia-inducible factors, or HIFs, induces the transcription of mitophagy-specific genes and mitophagy (Fig. 2) (12). Less well-characterized mTOR-independent regulators of autophagy also exist. One is ammonia, a by-product of amino acid catabolism, which stimulates autophagy, likely in poorly

perfused tissues and tumors (13). Glucagon, a predominant hormone of the fasted state, also triggers autophagy in the liver. Adrenergic receptor activation, which like glucagon activates adenylate cyclase and cyclic adenosine monophosphate (cAMP) production, also stimulates liver autophagy.

## Autophagy and Starvation

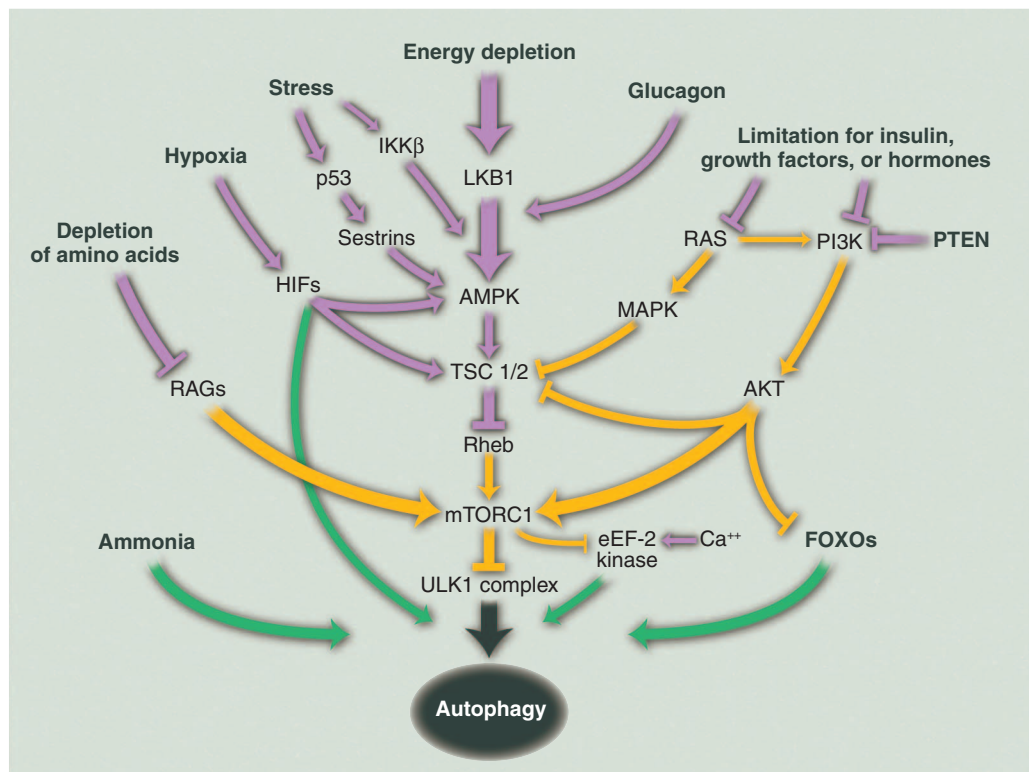
All cells have internal nutrient stores for use during starvation. Glycogen and lipid droplets are overtly designed for this purpose. Their contents are accessed primarily through the actions of dedicated enzymes, such as glycogen phosphorylase and hormone-sensitive lipase. Many other cellular components have a dual function as nutrient stores. For example, ribosomes occupy ~50% of the dry weight of rapidly growing microbes. In addition to enabling rapid protein synthesis when nutrient conditions are favorable, this provides a store of amino acids for proteome remodeling when conditions turn for the worse. Autophagy has a key role in providing access to such undedicated nutrient stores.

Limitation for any of the major elemental nutrients triggers autophagy in yeast, with nitrogen limitation the strongest stimulus (14). When ni-

trogen is removed, yeast defective in autophagy become severely depleted of internal amino acids. This precludes the synthesis of proteins important for surviving nitrogen starvation and accelerates cell death (15). Thus, autophagy provides the primary route to nitrogen during starvation.

Unlike microbes, mammalian cells benefit from a relatively constant nutrient environment. Nevertheless, autophagy can support mammalian cells through nutrient deprivation. For example, in lymphocytes, the ability to consume environmental nutrients is growth factor-dependent. In the absence of growth factor stimulation, energy charge is maintained through autophagy, with cells shrinking ~50% in size over 3 months of self-cannibalization (16).

At the organismal level, autophagy is required at multiple stages of mammalian development. The first directly follows oocyte fertilization, with autophagy essential to feed the developing embryo before it gains access to the maternal blood supply. Autophagy-defective embryos fail to reach the blastocyst stage (17). Maternally supplied autophagy proteins enable autophagy-deficient offspring to complete embryogenesis, revealing a second requirement for autophagy: when access



**Fig. 2.** Signaling pathways that regulate autophagy. Common nutrient, growth factor, hormone, and stress signals that regulate autophagy. Purple lines depict events that positively regulate autophagy. Yellow lines depict those that negatively regulate autophagy. Many pathways converge on the AMPK-mTORC1 axis. Green lines depict pathways that are mTOR-independent. Note that all input signals are framed as autophagy activators; thus, they include limitation for growth factors and nutrients. IKKβ, inhibitor of nuclear factor κB kinase β; PI3K, phosphatidylinositol-3 kinase; PTEN, phosphatase and tensin homolog; MAPK, mitogen-activated protein kinase; TSC1/2, tuberous sclerosis complexes 1 and 2; and EF, elongation factor.



to the maternal blood supply is suddenly lost due to birth. Autophagy-defective pups die within 24 hours of delivery. Both circulating and tissue amino acid levels are reduced, and AMPK is activated in the heart, which shows electrocardiographic changes analogous to those observed with severe myocardial infarction (18).

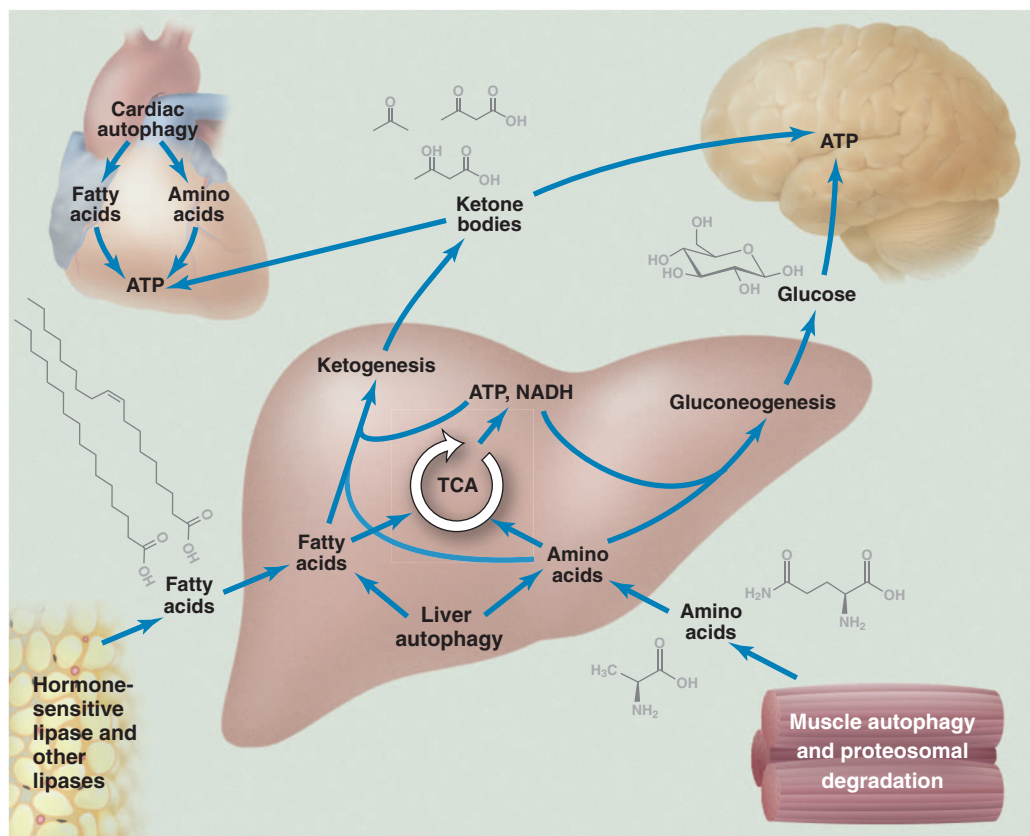
In adult starvation, autophagy also has a central role, increasing within 24 hours in liver, pancreas, kidney, skeletal muscle, and heart; the brain is spared (19). Pharmacological blockade of autophagy results in cardiac dysfunction early in starvation (20). Although autophagy levels return to normal in liver 2 days into starvation, it remains increased in both cardiac and skeletal muscle. Liver mass, however, persistently falls faster than muscle or total body mass. This decline is consistent with a failure of biosynthesis to balance basal consumption of liver by autophagy (21). As liver mass falls, breakdown of muscle and adipose tissue feeds the liver, which exports glucose and ketone bodies required by the brain (Fig. 3).

### Use of Metabolites Released by Autophagy

The breakdown products derived from autophagy have a dual role, providing substrates for both biosynthesis and energy generation (Fig. 1). In terms of biosynthesis, the abundance of ribosomal (relative to messenger) RNA makes transcriptome remodeling straightforward. In contrast, proteome remodeling demands copious amino acids, and a major role of autophagy is to provide them.

In addition to providing anabolic substrates, nucleosides and amino acids can be catabolized for energy generation. RNA breakdown yields nucleosides, which are degraded to ribose-phosphate. Six ribose-phosphate molecules are energetically equivalent to five glucose-phosphates, and, like glucose-phosphate derived by glycogen breakdown, they can yield adenosine triphosphate (ATP) either aerobically or anaerobically. In contrast, amino acids, like lipids, yield ATP only through oxidative phosphorylation (Fig. 1). The catastrophic effects of ischemia are a consequence of the relative paucity of nucleic acids and glycogen combined with the inefficiency of anaerobic glycolysis. Oxygen is the one nutrient that autophagy cannot provide.

In addition to being directly catabolized to yield energy, the liver can convert nucleosides, amino acids, and lipids into glucose and ketone



**Fig. 3.** Role of autophagy in adult mammalian starvation. Depicted pathways predominate after depletion of glycogen stores, typically ~12 hours into starvation. Autophagy in liver and heart (but not brain) generates fatty acids and amino acids, which are catabolized to yield energy. In the liver, this energy drives gluconeogenesis and ketogenesis. Amino acids are substrates for both ketogenesis and gluconeogenesis; acetyl-CoA from fatty acids is only for ketogenesis. As starvation continues, degradation of adipose and muscle play an increasing role in supplying substrates to the liver, which exports glucose and ketone bodies to feed the brain. The relative importance of ketone bodies increases in prolonged starvation. NADH, reduced form of nicotinamide adenine dinucleotide.

bodies for distribution elsewhere in the body (Fig. 3). Ribose-phosphate from nucleosides can be converted to glucose through the non-oxidative pentose phosphate pathway (PPP). Amino acids feed into central metabolism at multiple points, including pyruvate, tricarboxylic acid cycle (TCA) cycle intermediates, and acetyl-coenzyme A (CoA) (Fig. 1). Pyruvate and TCA cycle intermediates are substrates for gluconeogenesis. In contrast, mammals cannot convert acetyl-CoA into glucose. Because lipid degradation yields mostly acetyl-CoA, ketone bodies are essential for feeding the brain and other vital tissues during prolonged starvation.

### Autophagy as a Regulator of Metabolism

Autophagy is important for regulating cellular metabolic capabilities. A striking example comes from yeast capable of living off of methanol or fatty acids, substrates that are burned in peroxisomes. When more appealing forms of carbon become

available, the peroxisomes are no longer required and are cleared by autophagy (22). The function of autophagy in removing unneeded peroxisomes is conserved in mammals. Peroxisomes are induced in liver by various hydrophobic chemicals, known collectively as peroxisome proliferators. Removal of peroxisome proliferators leads to restoration of normal peroxisome abundance through autophagy (22).

Autophagy also regulates the abundance of liver lipid droplets by their constitutive degradation (23). Defective autophagy in mice leads to larger and more plentiful lipid droplets, increased concentrations of hepatic triglycerides and cholesterol, and increased gross liver size. Lipophagy is selectively decreased by free fatty acids in vitro and by a high-fat diet (23). Thus, in addition to promoting lipid droplet growth, free fatty acids may impair lipid droplet breakdown. Because lipophagy releases free fatty acids, its suppression by them is a case of one of the most prevalent regulatory motifs in metabolism: feedback inhibition. But this

feedback mechanism may backfire in the case of a chronic high-fat diet or obesity.

In contrast to the role of autophagy in clearing lipid droplets from the liver, autophagy is required for the production of the large lipid droplets characteristic of white adipose tissue (24, 25). White adipose refers to the canonical fat storage tissue that expands in obesity; this is in contrast to brown adipose, a mitochondria-rich tissue that catabolizes glucose and lipids to generate heat rather than ATP. Brown adipose tissue contains uncoupling protein 1, which allows protons to leak across the inner mitochondrial membrane, short-circuiting oxidative phosphorylation. Inhibition of autophagy blocks white adipocyte differentiation, and adipose-specific knockout of *atg7* results in mice whose white adipocytes manifest features typical of brown adipose tissue. Consistent with the rapid energy burning of brown adipocytes, these mice are lean; however, they are not healthy—when fed either a regular or high-fat diet, they are at increased risk of early death (24).

Autophagy also contributes to both insulin secretion and physiological sensitivity to the hormone. It is essential for the health of pancreatic  $\beta$  cells and for the expansion of  $\beta$ -cell mass that occurs in response to a high-fat diet (26, 27). In liver, defective autophagy leads to insulin resistance (28). For reasons that are not yet fully clear, hepatic autophagy is decreased in obese mice, and its restoration through retroviral expression of *atg7* ameliorates their insulin resistance.

## Overall Energetic Impact of Autophagy

To maintain homeostasis, tissue degradation by autophagy must be balanced by new macromolecule synthesis, which is energetically expensive. Each peptide bond in protein costs four high-energy phosphate bonds, two for tRNA charging and two for ribosome peptide bond formation. Assuming a free energy of ATP hydrolysis of  $-50$  kJ/mol under physiological conditions, synthesizing 1 g of protein consumes 1.8 kJ of energy. For a typical human, this means that rebuilding 10% of the body's protein content would consume at least 2000 kJ, or 20% of daily dietary energy intake. In vitro estimates of autophagic rates have generally been at or above the 10% per day level, for example, 1% of protein per hour for cultured hepatocytes (21). Accordingly, although in vivo rates of autophagy are presumably lower, rebuilding the structures degraded by autophagy may be a major contributor to mammalian caloric requirements. Consistent with this possibility, knockout of p62, which brings cargo to autophagosomes, results in decreased calorie burning and eventually obesity (29). During aging, both autophagy and total caloric expenditures decrease in tandem (30). The possibility of a causative link, in which decreased autophagy leads to reduced energy burned for self-regeneration, is intriguing. Decreased autophagy in obesity

may also contribute to the difficulty of losing weight (28).

## Autophagy and Disease

Cellular garbage disposal by autophagy prevents the buildup of damaged proteins and organelles that cause chronic tissue damage and disease. Genetic inactivation of autophagy in mice revealed that the type of disease depends on the tissue type. In the brain, autophagy suppresses the accumulation of ubiquitinated proteins, disposes of aggregation-prone proteins and damaged organelles that cause Huntington's and Parkinson's diseases, and prevents neurodegeneration (31, 32). In the liver, autophagy suppresses protein aggregate and lipid accumulation, oxidative stress, chronic cell death, inflammation, and cancer (4, 33). In intestinal Paneth cells, it preserves cellular function, prevents expression of damage and inflammatory markers, and prevents the development of Crohn's disease (34). Although the cell-refreshing role of autophagy functions in preventing the above diseases, autophagy's metabolic role may also contribute by ensuring consistent availability of internal nutrients and enabling cells to survive periods of poor external nutrition in good health. Regardless of the underlying mechanism, autophagy stimulation is under consideration for disease prevention. In support of this concept, autophagy mediates the protective effects of dietary restriction on aging-related diseases in model systems (35), and autophagy suppression contributes to the deleterious consequences of obesity (28). Induction of autophagy may result in the fasting rituals common in many religions as well as modern cleansing rituals, producing health benefits.

In contrast to normal cells in tissues, tumors often reside in an environment deprived of nutrients, growth factors, and oxygen as a result of insufficient or abnormal vascularization. Thus, the effects of autophagy in cancer are paradoxical: Although autophagy can prevent initiation of some cancers, it also may support tumor growth. Autophagy localizes to hypoxic tumor regions most distant from nutrient-supplying blood vessels, where it sustains tumor cell survival (36). Whether the primary role of autophagy in tumors is to provide metabolic substrates or prevent buildup of damaged components is not yet known. But either way, inhibition of autophagy may suppress the growth of established tumors (37).

Many of the pathways that control autophagy are deregulated in cancer (Fig. 2), and cancer therapeutics targeting these pathways activate autophagy. Some do so directly by inhibiting mTOR, whereas others inhibit upstream nutrient or signaling pathways. Cytotoxic cancer therapies activate autophagy, presumably by inflicting damage. The functional role of autophagy in these settings needs to be established. A particularly interesting possibility is that autophagy favors tumor cell survival. If this proves correct, then inhibition

of autophagy might synergize with existing cancer treatments (37).

## Conclusions

Autophagy is a major contributor to cellular metabolism. It provides internal nutrients when external ones are unavailable. It also provides an essential means of refreshing and remodeling cells. As such, it is required for normal development, including that of metabolic tissues such as adipose tissue and pancreatic  $\beta$  cells. In adults, autophagy promotes metabolic homeostasis and prevents degenerative disease and cancer. Once cancer occurs, however, autophagy may contribute to tumor resiliency. Thus, both activation and inhibition of autophagy hold promise for improved treatment of common, devastating diseases.

## References

1. A. Kuma, N. Mizushima, *Semin. Cell Dev. Biol.* **21**, 683 (2010).
2. S. F. Funderburk, Q. J. Wang, Z. Yue, *Trends Cell Biol.* **20**, 355 (2010).
3. D. L. Berry, E. H. Baehrecke, *Cell* **131**, 1137 (2007).
4. R. Mathew et al., *Cell* **137**, 1062 (2009).
5. T. Kanki, K. Wang, Y. Cao, M. Baba, D. J. Klionsky, *Dev. Cell* **17**, 98 (2009).
6. K. Okamoto, N. Kondo-Okamoto, Y. Ohsumi, *Dev. Cell* **17**, 87 (2009).
7. S. Geisler et al., *Nat. Cell Biol.* **12**, 119 (2010).
8. D. Narendra, A. Tanaka, D. F. Suen, R. J. Youle, *J. Cell Biol.* **183**, 795 (2008).
9. A. Efeyan, D. M. Sabatini, *Curr. Opin. Cell Biol.* **22**, 169 (2010).
10. D. B. Shackelford, R. J. Shaw, *Nat. Rev. Cancer* **9**, 563 (2009).
11. J. Zhao et al., *Cell Metab.* **6**, 472 (2007).
12. H. Zhang et al., *J. Biol. Chem.* **283**, 10892 (2008).
13. C. H. Eng, K. Yu, J. Lucas, E. White, R. T. Abraham, *Sci. Signal.* **3**, ra31 (2010).
14. K. Takeshige, M. Baba, S. Tsuboi, T. Noda, Y. Ohsumi, *J. Cell Biol.* **119**, 301 (1992).
15. J. Onodera, Y. Ohsumi, *J. Biol. Chem.* **280**, 31582 (2005).
16. J. J. Lum et al., *Cell* **120**, 237 (2005).
17. S. Tsukamoto et al., *Science* **321**, 117 (2008).
18. A. Kuma et al., *Nature* **432**, 1032 (2004).
19. N. Mizushima, A. Yamamoto, M. Matsui, T. Yoshimori, Y. Ohsumi, *Mol. Biol. Cell* **15**, 1101 (2004).
20. H. Kanamori et al., *Am. J. Pathol.* **174**, 1705 (2009).
21. M. Komatsu et al., *J. Cell Biol.* **169**, 425 (2005).
22. M. Oku, Y. Sakai, *FEBS J.* **277**, 3289 (2010).
23. R. Singh et al., *Nature* **458**, 1131 (2009).
24. R. Singh et al., *J. Clin. Invest.* **119**, 3329 (2009).
25. Y. Zhang et al., *Proc. Natl. Acad. Sci. U.S.A.* **106**, 19860 (2009).
26. C. Ebato et al., *Cell Metab.* **8**, 325 (2008).
27. H. S. Jung et al., *Cell Metab.* **8**, 318 (2008).
28. L. Yang, P. Li, S. Fu, E. S. Calay, G. S. Hotamisligil, *Cell Metab.* **11**, 467 (2010).
29. A. Rodriguez et al., *Cell Metab.* **3**, 211 (2006).
30. A. M. Cuervo, J. F. Dice, *J. Biol. Chem.* **275**, 31505 (2000).
31. T. Hara et al., *Nature* **441**, 885 (2006).
32. M. Komatsu et al., *Nature* **441**, 880 (2006).
33. M. Komatsu et al., *Cell* **131**, 1149 (2007).
34. K. Cadwell et al., *Nature* **456**, 259 (2008).
35. P. Kapahi et al., *Cell Metab.* **11**, 453 (2010).
36. K. Degenhardt et al., *Cancer Cell* **10**, 51 (2006).
37. E. White, R. S. DiPaola, *Clin. Cancer Res.* **15**, 5308 (2009).

10.1126/science.1193477



## REVIEW

# Circadian Integration of Metabolism and Energetics

Joseph Bass<sup>1,2,3,4,\*</sup> and Joseph S. Takahashi<sup>5,6</sup>

Circadian clocks align behavioral and biochemical processes with the day/night cycle. Nearly all vertebrate cells possess self-sustained clocks that couple endogenous rhythms with changes in cellular environment. Genetic disruption of clock genes in mice perturbs metabolic functions of specific tissues at distinct phases of the sleep/wake cycle. Circadian desynchrony, a characteristic of shift work and sleep disruption in humans, also leads to metabolic pathologies. Here, we review advances in understanding the interrelationship among circadian disruption, sleep deprivation, obesity, and diabetes and implications for rational therapeutics for these conditions.

The rising and setting of the sun has captivated naturalists interested in the cause of daily rhythmic phenomenon, prompting de Marian to demonstrate the existence of an internal clock in the 18th century by placing the *Mimosa* plant in a dark box and showing that its leaves continued to open and close every 24 hours. More than two centuries later, genetic studies in fruit flies paved the way for discovery that the circadian clock is encoded by a set of transcriptional activators and repressors that comprise an autoregulatory transcription-translation feedback loop. The circadian clock in mammals is expressed within pacemaker neurons of the suprachiasmatic nucleus (SCN) that in turn maintain proper phase alignment of peripheral tissue clocks present in nearly all cells. Thus, the brain SCN clock provides “standard time” for all peripheral tissue clocks. In experimental models, clock disruption leads to disorders in glucose metabolism, confirming a role for these genes as key regulators of metabolism and supporting the hypothesis proposed by McKnight and colleagues that circadian cycles are intimately interconnected with metabolic cycles (1). Accumulating evidence has revealed that multiple clock genes participate in metabolic homeostasis, suggesting that these proteins have evolved overlapping (or convergent) functions both as intrinsic “hands” of the clock and as regulators of metabolism. Although still at an early stage, emerging studies in humans suggest parallels in the role of circadian genes and metabolic homeostasis. At the epidemiological

level, it has been suggested that increased activity during what was “rest” time in the premodern world, together with sleep disruption, have been associated with an increased prevalence of obesity, diabetes, and cardiovascular disease, in addition to certain cancers and inflammatory disorders. This review highlights advances in understanding the molecular coupling between metabolic and clock networks and its relevance to gene-environment and brain-behavioral systems important in energy balance and metabolic disease.

## Core Transcriptional Components of the Clock and Posttranslational Regulation

Features of the circadian clock in all organisms include its persistence under constant conditions, a periodicity that is temperature compensated, and its entrainment to light from the sun (Fig. 1). In mammals, cell-autonomous circadian clocks are generated by a transcriptional autoregulatory feedback loop composed of the transcriptional activators CLOCK and BMAL1 and their target genes *Period* (*Per*) and *Cryptochrome* (*Cry*), which rhythmically accumulate and form a repressor complex that interacts with CLOCK-BMAL1 to inhibit their own transcription (2). This autoregulatory loop is posttranscriptionally regulated by casein kinases (CK1 $\epsilon$  and CK1 $\delta$ ), which target the PER proteins for degradation via the Skp1, Cullin1, F-box protein (SCF)/ $\beta$ -TrCP ubiquitin ligase complex, and by adenosine monophosphate (AMP) kinase, which targets the CRY proteins for degradation via the SCF/FBXL3 ubiquitin ligase complex by the 26S proteasome [reviewed in (2)] (Fig. 2).

The prevailing model of the circadian clock involves the transcription-translation feedback loop, but less is known about nontranscriptional mechanisms that may generate circadian oscillations. In cyanobacteria, cycles of protein phosphorylation are sufficient to generate biological rhythms in the absence of transcription (3). In the mammalian SCN, changes in cyclic AMP levels alter period length, an additional example

of posttranslational signaling as a mechanism controlling of circadian cycles (4), and recent work has shown that the SCN neuronal coupling network itself has intrinsic oscillatory function that can emerge in the absence of cell-autonomous oscillators (5). RNA interference screening of mammalian cells also indicates coupling of the peripheral clock to phosphatidylinositol 3-kinase signaling (6). Further research is warranted to elucidate the impact of posttranslational signaling pathways on the core clock and its physiological outputs.

Fibroblast cell lines display ~24 hours oscillation of core clock genes, demonstrating that the clock is expressed not only in neurons but also in peripheral tissues (7). Intrinsic oscillation of clocks in liver cells can be entrained by food, whereas oscillation of the brain clock is resilient and entrained primarily by light (8). A recurring theme in understanding the coupling between circadian and metabolic systems is the recognition that the two systems are reciprocally regulated; food entrains the liver clock, whereas light acts through the brain clock to control feeding time.

## Crosstalk Between the Clock and Metabolic Transcription Networks

*Nuclear hormone receptors and the phase alignment of metabolic gene expression cycles.* Direct evidence for metabolic input into the core clock includes the finding that the orphan nuclear hormone receptor (NHR) reverse-erb alpha (REV-ERB $\alpha$ ) (a repressor) and the opposing retinoic acid orphan receptors (ROR $\alpha$  and  $\beta$ ) (activators) constitute a short feedback loop controlling *Bmal1* transcription (9, 10) (Fig. 2). Peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) and the coactivator peroxisome proliferators-activated receptor gamma coactivator 1- $\alpha$  (PGC1 $\alpha$ ) also modulate *Bmal1* transcription through this feedback loop (11), indicating that REV-ERB $\alpha$  is a nodal point for metabolic input into the clock. NHR profiling has revealed rhythmic clustering of these factors in metabolic tissues across the day/night cycle, suggesting extensive coupling between circadian and nuclear receptor signaling networks (12). These findings raise the possibility that disruption of NHR cycles may perturb the clock and, conversely, that delay, advance, or reduced amplitude of circadian oscillations may impair NHR function. Knock-in mice of the NHR co-repressor NCor display increased energy expenditure and a shift in the oscillation in the abundance of RNAs encoding oxidative, glycolytic, and respiratory genes, indicating that disruption of the phase of expression of NHRs contributes to metabolic dysregulation (13). Mistiming of gene expression rhythms as a cause of metabolic dysregulation has also been suggested by studies in *Rev-erba* mutant animals, in which a phase shift in oscillating rhythms of metabolic gene transcription, rather than changes in total abundance of RNA, correspond with altered

<sup>1</sup>Department of Medicine, Northwestern University, Feinberg School of Medicine, Chicago, IL 60611, USA. <sup>2</sup>Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208–3520, USA. <sup>3</sup>Weinberg College of Arts and Sciences, Northwestern University, Evanston, IL 60208–3520, USA. <sup>4</sup>Center for Sleep and Circadian Biology, Northwestern University, Evanston, IL 60208–3520, USA. <sup>5</sup>Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX 75390–9111, USA. <sup>6</sup>Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX 75390–9111, USA.

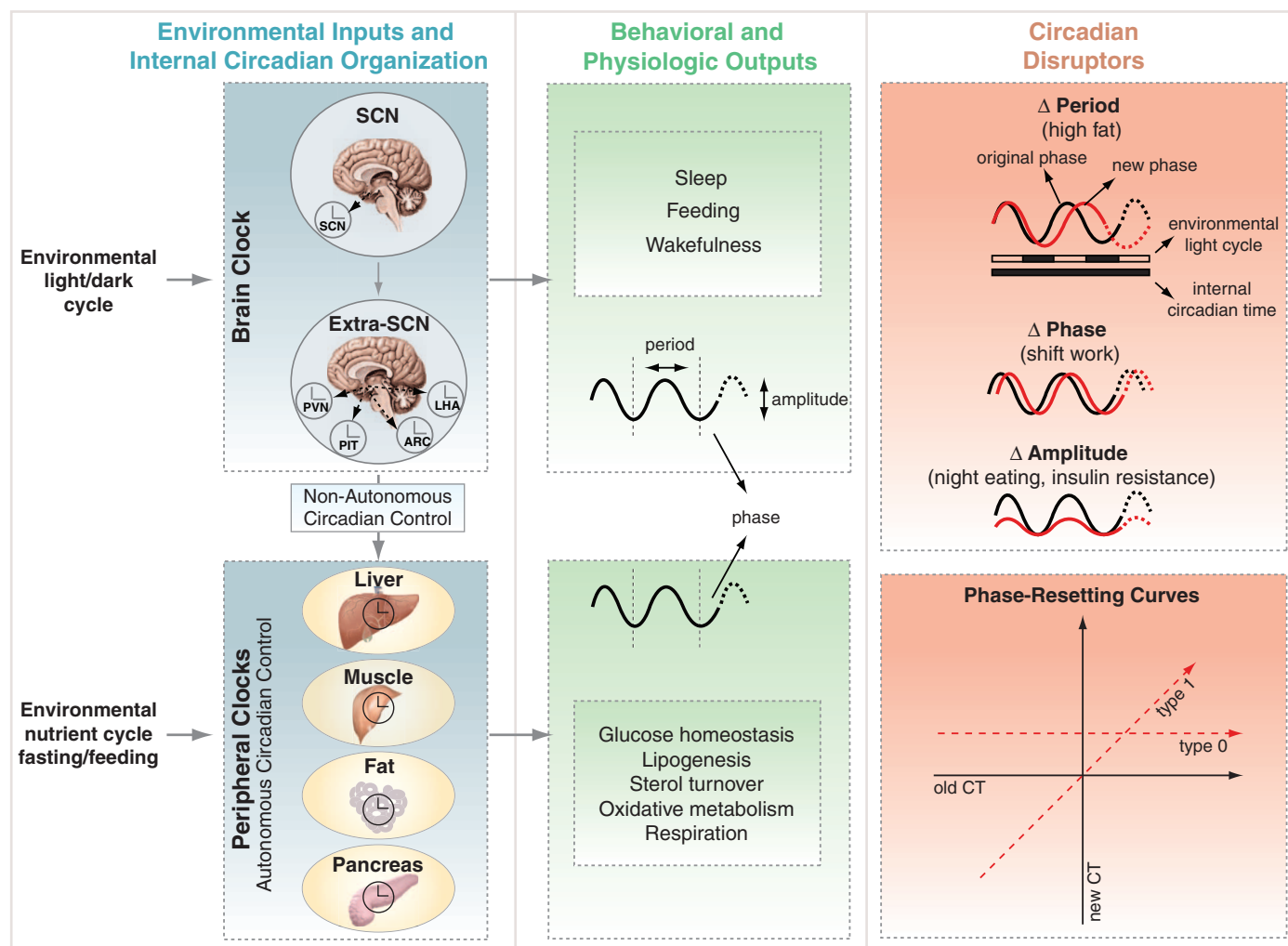
\*To whom correspondence should be addressed. E-mail: j-bass@northwestern.edu

energy balance (14). Misalignment between gene transcription cycles within metabolic tissues and the behavioral cycle (of fasting and feeding) may be sufficient to alter energy homeostasis. For instance, high-fat feeding provided at the incorrect circadian time leads to greater weight gain in mice than isocaloric feeding at the normal circadian time (15).

*Direct versus indirect role of clock transcription factors in metabolic gene regulation.* It is likely that disruption within the core clock may be transmitted to metabolic outputs through alterations in NHRs or directly by actions of clock activators or repressors. For example, PER2 directly occupies promoters of certain metabolic

genes (16). Alternatively, the clock activator loop drives D-element-binding protein expression, providing indirect regulation of gluconeogenic genes (17). This raises the question as to whether the effects of clock-gene disruption relate to direct alterations in “timing” per se or to indirect effects arising from independent activity of the clock factors on metabolic networks. The dichotomy between circadian versus noncircadian actions of clock proteins may not be fully valid, because the rhythmic abundance in the expression level of these proteins in turn may produce rhythmic changes in metabolism. For example, CRY, a rhythmically expressed clock repressor, modulates gluconeogenesis through interference with

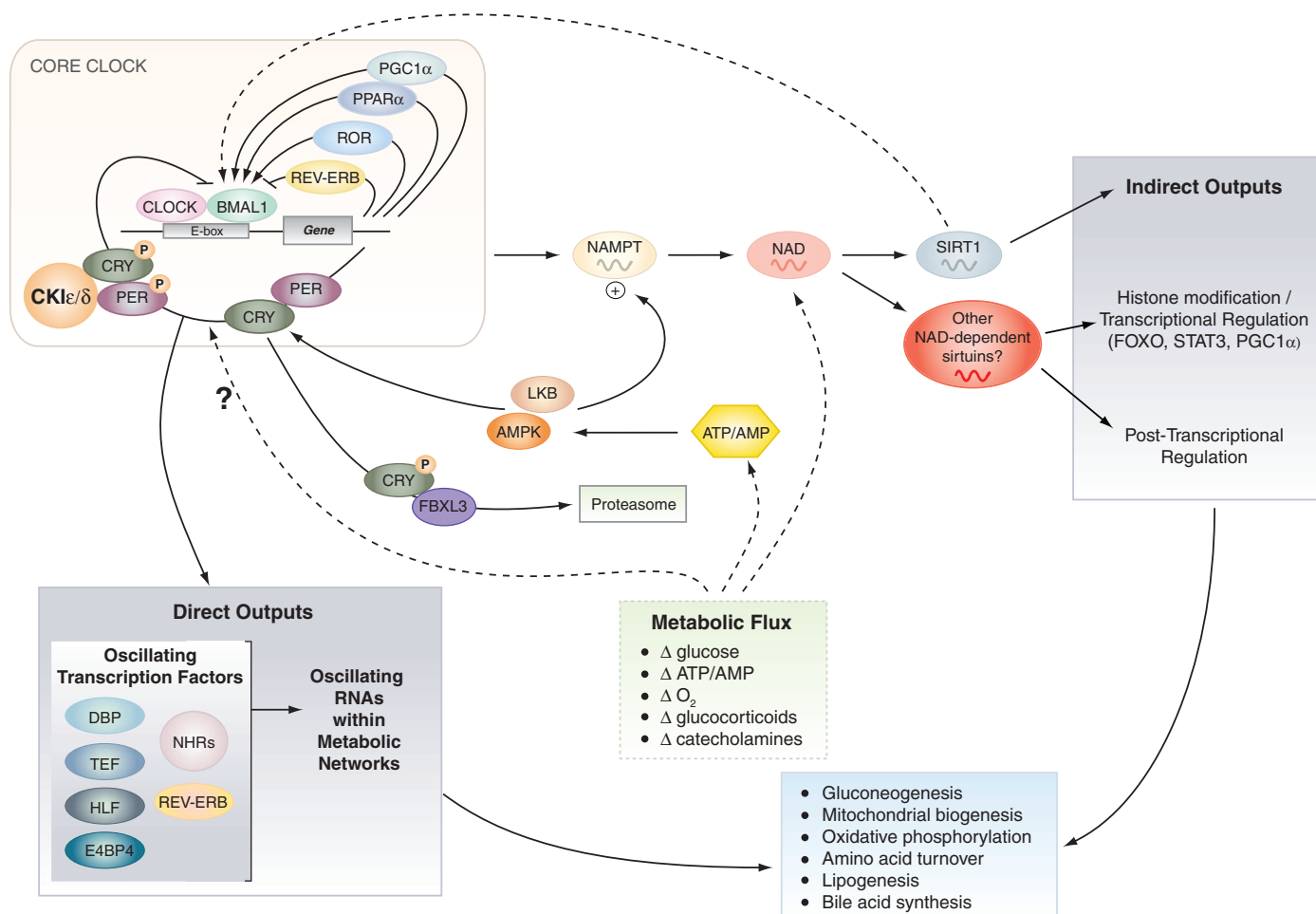
glucagon and inhibition of cyclic AMP signaling (18). Conceptually, the question of timing versus expression as a cause of metabolic disorders after disruption of clock genes is akin to the difference between a musical performer playing the wrong notes or playing the right notes at the wrong time. One experimental approach to tease apart the role of circadian timing per se in physiology would be to investigate whether physiological defects could be corrected by alignment of the internal period with the external light cycle (i.e., a test of “resonance”). In plants, various period-length mutants have improved photosynthesis and growth when exposed to external light cycles that matched the endogenous circadian period (19).



**Fig. 1.** Central and peripheral clocks coordinate external cues with behavior and metabolic outputs. Light entrains the master pacemaker in the SCN, which in turn synchronizes extra-SCN and peripheral clocks. Brain clock outputs include behavioral rhythms (i.e., sleep and feeding), whereas peripheral clock outputs include metabolic rhythms (e.g., glucose and lipid homeostasis). The hierarchical organization of the mammalian clock is highlighted, with “nonautonomous” regulation of peripheral tissue clocks denoting the regulation of peripheral tissue oscillators through direct neural and humoral signals, and “autonomous” regulation indicating the intrinsic regulation of local cellular oscillators independently of the brain clock. Highlighted to the

right are the three possible ways to disrupt the clock by changing period, phase, or amplitude, each of which can trigger disorders of metabolism. Phase resetting can be broadly classified into two groups based on phase response after delivery of the agent at sequential time points across the 24-hour cycle. Type 1 or weak resetting indicates that the slope of the plot relating the new to the old circadian phase is 1 (interventions that cause different phase shifts at different circadian times). Type 0 or strong resetting indicates that the slope of the new to the old circadian phase is 0 (i.e., interventions that cause the same phase at all circadian times). PVN, paraventricular nucleus; PIT, pituitary; ARC, arcuate nucleus; LHA, lateral hypothalamic area.





**Fig. 2.** Direct and indirect outputs of the core clock mechanism. The core clock consists of a series of transcription/translation feedback loops that synchronize diverse metabolic processes through both direct and indirect outputs, including

gluconeogenesis and oxidative metabolism. The clock also receives reciprocal input from nutrient signaling pathways (including *SIRT1* and *AMPK*), which function as rheostats to couple circadian cycles to metabolic flux, especially in peripheral tissues.

**Reciprocal control of clock by metabolic signaling.** An intriguing question remains the extent to which NHRs modulate circadian systems according to changing environmental conditions, such as humoral or nutritional factors. For example, variation in the concentration of glucocorticoid hormone, retinoic acid, heme, and fatty acids affect glucocorticoid receptors (GRs), retinoic acid receptor (RAR), *REV-ERBα*, neuronal Per-Arnt-Sim (PAS) domain protein 2 (NPAS2), and PPARs. Therefore, variation in cellular concentrations of any one of these ligands might influence *Bmal1* transcription and thereby modulate local cellular circadian rhythms. Within brain, heme and carbon monoxide may modulate NPAS2 activity (20), whereas within vascular cells, retinoic acid influences circadian oscillations through activation of *RARα* and *RXRα* (21). Likewise, rhythmic variation in NHR ligands may exert distinct effects on local tissue clock function at different times in the day/night cycle.

Local differences in NHR expression may give rise to tissue-specific differences in coupling

of circadian and metabolic cycles, although this remains to be tested. For example, *PER2* forms physical interactions with *PPARα* and *REV-ERBα* (16), in turn modulating transcription of the gluconeogenic factor *G6pase*. Conversely, oscillation in NHR ligands may affect not only the phase and amplitude of circadian rhythms but also physiological outputs of the circadian system. For example, glucocorticoid receptor binding to the promoter of *PER2* modulates leptin production and glucose tolerance (22). Non-autonomous signals, such as glucocorticoids or other systemic cues, may have an especially important role in sustaining oscillations of *PER2* even in the absence of rhythmic oscillation of *CLOCK*-*BMAL1* (23). It may be possible to exploit tissue-specific differences in NHR-clock interactions for therapeutic purposes.

NHRs may also participate in entrainment of central and peripheral clocks. In vertebrates, a hierarchy of signals within SCN pacemaker neurons in the brain and downstream extra-SCN neurons generates entraining cues to maintain phase alignment between oscillators in multiple peripheral

tissues. An unresolved question is whether peripheral organ clocks are principally entrained through direct neural wiring or through circulating hormones, such as glucocorticoids (24). The impact of glucocorticoids on hepatic entrainment has important implications for health under conditions of circadian misalignment, such as phase resetting during jet lag or shift work (25). The finding that liver and brain respond to different entraining signals points toward a possible weak point in the system; conditions such as insulin resistance, where signaling through glucocorticoid, catecholaminergic, or peptideric hormones may be attenuated, may cause misalignment between the phase of central and peripheral oscillators. A related phenomenon is food anticipatory activity (FAA) caused by food presentation at the incorrect circadian time. Although clock-gene function in FAA behavior has been debated, abrogation of melanocortineric signaling influences this behavior, consistent with a noncircadian timing mechanism (26). Finally, body temperature has been shown to be a powerful entraining agent for peripheral oscillators. Indeed, most signals that

synchronize peripheral oscillators affect either body temperature or the heat shock pathway, so this may be a final common pathway for resetting clocks in mammals (27).

## How Circadian Disruption Causes Metabolic Pathologies: Experimental Genetic Models and Human Clinical Studies

**REV-ERB and bile acid synthesis.** Further studies on the repressor REV-ERB $\alpha$  have uncovered a connection between the clock and the master pathway of hepatic lipid metabolism involving the sterol regulatory element-binding protein (SREBP). SREBPs control both fatty acid and sterol biosynthesis through modulation of rate-limiting enzymes in these pathways. Using a combination of genetic loss and gain of function approaches, Le Martelot *et al.* observed that the nuclear receptor REV-ERB $\alpha$  controls oscillation in the abundance and activation of the SREBPs through modulation of the enzyme INSIG2 (insulin-induced gene 2), an insulin-responsive factor that regulates SREBP release from the endoplasmic reticulum. REV-ERB $\alpha$  knockout mice develop increased lipogenesis through up-regulation of SREBP1c and SREBP2 target genes independently of nutrient state. In contrast, REV-ERB $\alpha$ -overexpressing mice have decreased SREBP target gene transcription and correspondingly reduced circulating lipid concentrations. The effects of REV-ERB $\alpha$  on bile acid metabolism are mediated through alteration of oxysterol synthesis and liver X receptor (LXR) activity (14), although effects of the transcription factor, the small heterodimeric protein (SHP), and E4BP4 (adenoviral E4 protein-binding protein) have also been implicated in this process (28). Because REV-ERB $\alpha$  expression is controlled by CLOCK-BMAL1, the rhythmic regulation of bile acid production may represent one of the first direct molecular outputs of circadian clock on metabolic physiology.

**Clock network and lipogenesis.** In addition to clock control of bile acid synthesis, mounting evidence has implicated both a direct and indirect effect of clock transcription factors on other aspects of lipogenesis. These observations stem from the finding that *Clock* <sup>$\Delta$ 19</sup> mutant mice develop hypertriglyceridemia (29) due to effects within both enterocytes and liver (30). At the level of the intestine, the clock regulates triglyceride packaging into chylomicrons (globules that transport dietary lipids), whereas in the liver, clock disruption triggers lipid accumulation (30). The clock-controlled gene *Nocturnin* also affects the inter-related processes of lipogenesis, osteogenesis (bone formation), and energy homeostasis (31–33). Effects of circadian gene mutations on lipid absorption are strain-dependent in mice, with severe steatorrhea (excess fecal fat) and malabsorption occurring in the ICR (Institute for Cancer Research) strain, thereby masking the effects of the *Clock* <sup>$\Delta$ 19</sup> mutation on hepatic triglyceride production, and diet-induced obesity (34).

The clock also functions in ultradian variation (cycles occurring multiple times in 24 hours) of endoplasmic reticulum (ER) stress signaling, which in turn modulates SREBP activation through a posttranscriptional pathway (35). Rhythmic oscillation of phosphorylation of inositol-requiring enzyme 1 $\alpha$  (IRE1 $\alpha$ ), a transducer of the ER stress response, triggers rhythmic cleavage and translocation of SREBP into the nucleus. The ER stress response detects unfolded or improperly folded proteins; thus, rhythmic activation of IRE1 $\alpha$  integrates circadian, stress-signaling, and lipogenic pathways. Indeed, ultradian rhythms within liver appear with a 12-hour periodicity in the expression of many clock-controlled RNAs (36) and even in rhythmic oscillation of the metabolite nicotinamide adenine dinucleotide (NAD<sup>+</sup>). These shorter cycles are harmonics of the 24-hour cycle and may in turn produce rhythmic patterns in physiologic pathways such as lipogenesis. In *Cry1/Cry2* double-knockout mice, which harbor disruption within the repressor limb of the core clock, loss of circadian oscillation corresponded with constitutive IRE1 $\alpha$  activation and accumulation of hepatic lipids. In contrast, in *Clock* <sup>$\Delta$ 19</sup> mutant mice, which are deficient in the clock activation limb, there were opposite effects on SREBP activation. The finding that ablation of activators and repressors each produce physiologic effects builds evidence that lipogenesis is driven by the circadian clock rather than an epiphenomenon of clock-gene disruption.

The aforementioned studies in mice also have implications for metabolic functions of the clock in humans, because clock genes oscillate within human adipocytes (37) and alterations in clock-gene expression are correlated with obesity (38). These findings increase the need to delineate the relationship between chronotype (e.g., whether one is a “lark” or a “night owl”), clock genotype, and metabolic physiology in humans.

**Circadian regulation of cardiovascular function, inflammation, and thrombosis.** It is axiomatic in clinical medicine that certain cardiovascular catastrophes, including myocardial infarction and thrombosis, cluster early in the morning. Yet, the mechanistic underpinnings of timing in cardiovascular disease are not understood. Many aspects of fatty acid metabolism, a key fuel for cardiac muscle, exhibit strong circadian variation, and clock disruption affects chronotropic function (39). Ablation of *Bmal1* also increases the extent of arterial wall lesions after endothelial injury (40), suggesting multiple ways through which clock genes may influence susceptibility to myocardial damage. Similarly, autonomic and mineralocorticoid control of vascular tone, factors in cardiovascular disease risk, have been tied to the clock (41, 42). In individuals with metabolic syndrome, one predictor of cardiovascular risk is the absence of normal nocturnal variation in blood pressure, so-called “nondippers” (43). Production of the prothrombotic molecule plas-

minogen activation inhibitor-1 (PAI-1) has been shown to exhibit circadian regulation (44, 45). Thus, inflammation, thrombosis, cardiomyocyte metabolism, vascular tone, and response to vascular injury each represent phenotypes affected by circadian clock function.

**Circadian systems in glucose homeostasis and diabetes.** Glucose concentrations in the blood are highly rhythmic because of changes in insulin sensitivity and insulin secretory capacity of endocrine pancreas (46). Individuals with type 2 diabetes, and even their first-degree relatives not yet affected with the disease, display altered rhythmicity in glucose tolerance (47). Although early morning insulin resistance has been ascribed to the surge in growth hormone during slow-wave sleep, rhythmic variation in insulin sensitivity is in part due to autonomic rhythms generated by afferent input from hypothalamus to liver, downstream of the circadian clock (48). Ever since the inception of insulin use in clinical practice, recapitulating the endogenous rhythm of insulin production, and achieving a proper match in the variation in insulin requirement throughout the day/night cycle, has been a pragmatic clinical goal.

Rhythmic production of insulin regulated by peripheral  $\beta$ -cell clocks was revealed by continuous perfusion of isolated islets from the rat, which has a 24-hour rhythm (49). Live-cell imaging in islets isolated from *Per2-Luciferase* mice shows that they express a self-sustained oscillator with period length matching that of the liver and pituitary (50). *Clock* <sup>$\Delta$ 19</sup> mutant mice develop age-dependent hyperglycemia in both the light and dark phases of the cycle, corresponding with periods of fasting and feeding (29). These animals also develop susceptibility to diet-induced obesity; however, rather than displaying the anticipated hyperinsulinemia, they instead have inappropriately low concentrations of insulin. *Clock* <sup>$\Delta$ 19</sup> mutant mice display a steeper drop in blood sugar in response to treatment with insulin, a sign that these animals have enhanced insulin sensitivity, thereby masking their  $\beta$ -cell deficiency (50). *Bmal1* mutant mice also have impaired glucose tolerance (51), increased insulin sensitivity, and a progressive myopathy with aging that causes cachexia, which limits interpretation of glucose turnover studies. Studies in isolated islets revealed first that the clock oscillator is expressed and self-sustained in this tissue and, second, that glucose responsiveness in islets is diminished when the clock is disrupted. After middle age, the mutants also have smaller islet size, reduced proliferation, and transcriptome-wide decreases in proliferative gene expression. Studies in tissue-specific knockout mice have supported the hypothesis that function of the clock activators in the liver opposes their function in the pancreas (51). Whereas ablation of *Bmal1* exclusively within the islet does not affect activity behavior, feeding, or body weight, these mice display a much



greater impairment of glucose tolerance than the global knockout, as predicted. Islets from both global and pancreas-specific knockouts have normal insulin content, and influx of calcium in response to glucose is intact. However, exocytosis is impaired, suggesting that the clock controls the latest stage in stimulus-secretion coupling.

Findings in experimental genetic models of clock-gene ablation may also have implications for understanding emerging evidence that the circadian system participates in human glucose metabolism. For instance, in genome-wide association studies, variation in the *Melatonin 1b receptor* (*MTNR1B*) and in *Cry2* are both associated with blood glucose concentrations [(52) and reviewed in (53)]. *MTNR1B*, the cognate receptor of the circadian-regulated hormone melatonin, is expressed in many metabolic tissues, whereas *Cry2* encodes a clock repressor. These findings underscore the need to incorporate temporal considerations at the planning stages in future studies to account for circadian variation. Similarly, temporal considerations may aid in analysis of experimental genetic models because testing at different times and under different environmental light cycles may uncover unanticipated effects.

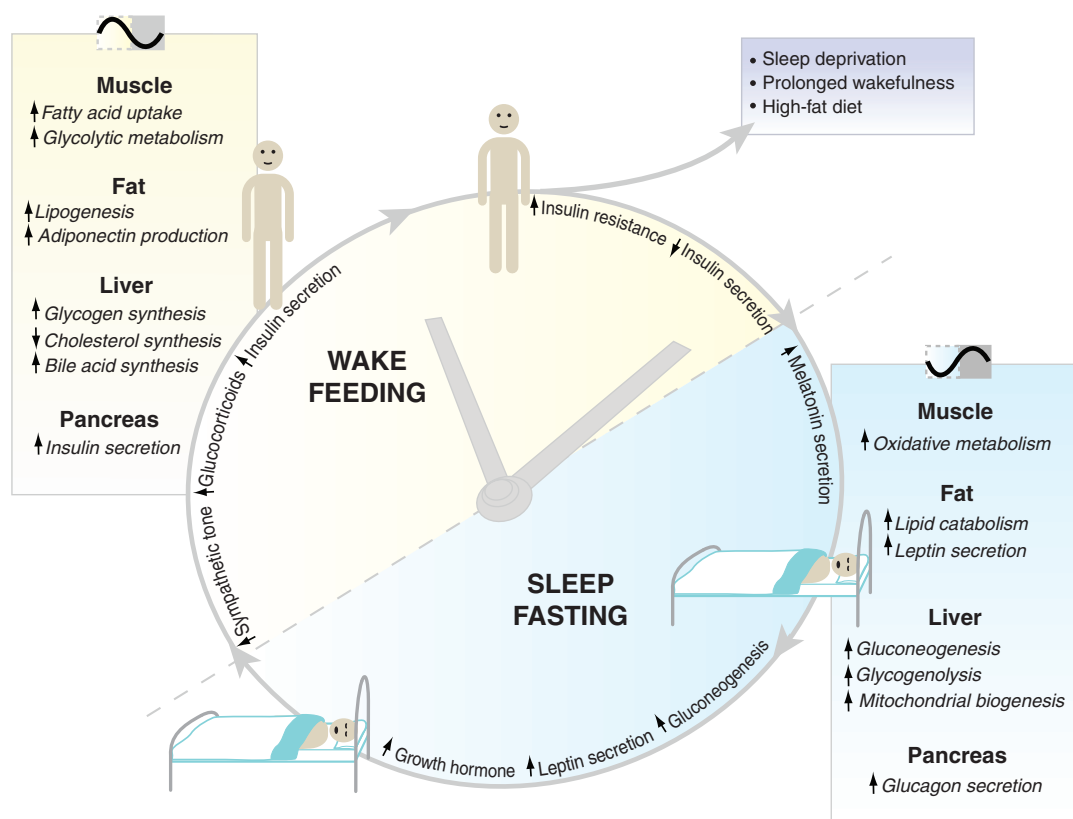
**Sleep and forced circadian misalignment: genetic models and human studies.** Ties between circadian disruption and metabolic disturbance have garnered attention, including large cross-sectional sampling of populations subjected to shift work. Extensive studies also indicate a correlation between sleep time and body mass index (BMI). Disruption in specific phases of sleep may be connected to metabolic function. Subtle tones sufficient to selectively deprive subjects of slow-wave sleep without producing conscious wakefulness were sufficient to impair glucose tolerance (54). Neuroanatomic studies also indicate interconnections between regions of hypothalamus important in circadian signaling, energetics, and sleep (55, 56). At the molecular level, orexin (also termed hypocretin), originally discovered as a neuropeptide produced in the feeding-stimulatory neurons of lateral hypothalamus, is positioned at the intersection of neuronal systems controlling sleep, circadian output, and metabolism (56). Analysis of orexin receptor 2 knockout mice

indicates that lack of orexin signaling increases susceptibility to obesity (rather than the original expectation that orexin, a potent wakefulness-inducing peptide, would induce adiposity) (57). Orexin receptor 2 mutations also account for canine narcolepsy, and orexin deficiency is a hallmark of the disease in humans (58). Activity of the orexin neuron is modulated by glucose and integrates signals downstream of leptin-responsive neurons within the arcuate nucleus. Leptin also affects sleep, possibly independently of effects on body weight, raising the need to further define leptin actions in this process (59). Manipulation of orexin signaling, an integrator of energetic and

pupulation that is intended to simulate deleterious effects of jet lag or shift work, caused impaired glucose tolerance and hypoleptinemia. Whether circadian disruption might also affect endocrine pancreas insulin secretion, hepatic gluconeogenesis, and glucose disposal in skeletal muscle in humans awaits further study; however, these results emphasize the clinical linkages between circadian function and metabolic homeostasis.

### Coupling and Outputs: How Do Clocks Sense and Respond to Nutrient Signals?

Under homeostatic conditions, the clock acts as a driver of metabolic physiology (Fig. 3). However,



**Fig. 3.** The clock partitions behavioral and metabolic processes according to time of day. The clock coordinates appropriate metabolic responses within peripheral tissues with the light/dark cycle. For example, the liver clock promotes gluconeogenesis and glycogenolysis during the sleep/fasting period, whereas it promotes glycogen and cholesterol synthesis during the wake/feeding period. Proper functioning of peripheral clocks keeps metabolic processes in synchrony with the environment, which is critical for maintaining health of the organism. Different tissues exhibit distinct clock-controlled properties; thus, ablation of the clock in certain tissues will cause opposing effects on metabolic function as uncovered through dynamic challenges at different times in the cycle under different nutrient conditions. Aging, diet, and environmental disruption such as shift work may also affect the integration of circadian and metabolic systems.

circadian signals, may thus provide opportunities to intervene not only in disorders of sleep but also in related metabolic complications.

In humans exposed to a light/dark cycle lengthened to 28 hours, out of synchrony with the endogenous clock, the sleep/wake cycle is driven at 28 hours, whereas the melatonin and body-temperature rhythm free-runs with a ~24-hour period (60). Such “forced desynchrony,” a mani-

with perturbations in either circadian or metabolic systems, such as forced behavioral misalignment with shift work or, conversely, high-fat feeding, a vicious cycle ensues in which disruption of metabolic pathways damp and lengthen circadian oscillations (61). The identity of metabolic sensors that may act as intermediates in coupling circadian cycles with physiologic systems remains to be identified. For instance, do changes in cell-

nutrient signaling in turn produce changes in circadian clock function? Does metabolic disease lead to altered amplitude or phase of circadian cycles within brain or peripheral organs? Two lines of research have begun to address these questions: first, involving the cellular pathway of AMP concentrations, and second, involving NAD<sup>+</sup> metabolism. Using phosphopeptide mapping, Lamia *et al.* identified a consensus motif for phosphorylation by AMP-activated protein kinase (AMPK), a sensor of AMP/ATP (adenosine triphosphate) ratio, within the CRY protein (62). AMP kinase activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) promoted degradation of CRY, which was abrogated by mutation of the AMPK consensus motif. AMPK knockout mouse embryonic fibroblasts (MEFs) also displayed altered rhythmicity, leading to the proposal that AMP concentration directly couples circadian rhythms to nutrient state in peripheral cells.

A second example in metabolic coupling with the core clock originated with the finding that the mammalian ortholog of the yeast sirtuin deacetylases (for silent information regulator)—proteins that activate or silence chromatin according to availability of fuel—comprises part of an additional feedback loop with the core clock (63, 64). Sirtuins are present in transcription complexes with the clock and in turn modulate activity of clock transcription factors. CLOCK-BMAL1 activates the major pathway for mammalian NAD<sup>+</sup> synthesis, involving its regeneration from nicotinamide mediated by nicotinamide phosphoribosyltransferase (NAMPT) (65) (66). NAD<sup>+</sup> concentration in cells varies across the light/dark cycle, consistent with a role for NAD<sup>+</sup> as an oscillating metabolite linking metabolic cycles with the clock. Both NAMPT and SIRT1, similar to the NHRs and AMPK, are regulated not only by the clock but also by the nutritional status of the organism. For example, fasting increases NAMPT expression in an AMPK-dependent manner in skeletal muscle, whereas fasting and caloric restriction increase SIRT1 activity across multiple tissues. Thus, regulation of the clock by NAD<sup>+</sup> and SIRT1 allows for fine-tuning and synchronization of the core molecular clock with the environment. Because NAD<sup>+</sup>-dependent deacetylases regulate gluconeogenesis and many other pathways (67), it will be important to further delineate the role of the clock in NAD<sup>+</sup>-driven metabolism (Fig. 2). A second NAD<sup>+</sup>-regulated pathway has recently been linked to circadian feeding cycles: PARP-1 activity is circadian in the liver, causing the rhythmic addition of poly-adenosine-diphosphate-ribose residues to the CLOCK protein. Because PARP-1 is regulated by NAD<sup>+</sup>, this provides yet another pathway for metabolic signals to regulate the core clock pathway (68). Collectively, these findings identify incoming (AMPK and PARP-1) and outgoing (NAD<sup>+</sup>/Sirtuin) sensors

that couple nutrient availability, metabolism, and the clock.

## Conclusion

In just the past 20 years, the mystery of biological timing has been transformed through genetic discovery. As a consequence, the availability of molecular clock genes has now provided tools to understand the physiological functions of the circadian system in unprecedented detail. As we experimentally dismantle the clock, the interdependence of timing and energetics seems inextricable. Major gaps in our understanding include (i) the connection between brain and peripheral tissue clocks in metabolic homeostasis, (ii) the interplay between circadian and sleep disruption in energetics, (iii) the relationship between nutrient state and circadian homeostasis, and (iv) the impact of circadian clock systems on human physiology. Ultimately, such studies will yield deeper insight into the interconnections between genes, behavior, and metabolic disease.

## References and Notes

1. J. Rutter, M. Reick, S. L. McKnight, *Annu. Rev. Biochem.* **71**, 307 (2002).
2. J. S. Takahashi, H. K. Hong, C. H. Ko, E. L. McDearmon, *Nat. Rev. Genet.* **9**, 764 (2008).
3. M. Nakajima *et al.*, *Science* **308**, 414 (2005).
4. J. S. O'Neill, E. S. Maywood, J. E. Chesham, J. S. Takahashi, M. H. Hastings, *Science* **320**, 949 (2008).
5. C. H. Ko *et al.*, *PLoS Biol.* **8**, e1000513 (2010).
6. E. Zhang *et al.*, *Cell* **139**, 199 (2009).
7. A. Balsalobre, F. Damiola, U. Schibler, *Cell* **93**, 929 (1998).
8. K. A. Stokkan, S. Yamazaki, H. Tei, Y. Sakaki, M. Menaker, *Science* **291**, 490 (2001).
9. N. Preitner *et al.*, *Cell* **110**, 251 (2002).
10. H. Duez, B. Staels, *Diab. Vasc. Dis. Res.* **5**, 82 (2008).
11. C. Liu, S. Li, T. Liu, J. Borjigin, J. D. Lin, *Nature* **447**, 477 (2007).
12. X. Yang *et al.*, *Cell* **126**, 801 (2006).
13. T. Alenghat *et al.*, *Nature* **456**, 997 (2008).
14. G. Le Martelot *et al.*, *PLoS Biol.* **7**, e1000181 (2009).
15. D. M. Arble, J. Bass, A. D. Laposky, M. H. Vitaterna, F. W. Turek, *Obesity (Silver Spring)* **17**, 2100 (2009).
16. I. Schmutz, J. A. Ripberger, S. Baeriswyl-Aebischer, U. Albrecht, *Genes Dev.* **24**, 345 (2010).
17. W. J. Roesler, P. J. McFie, C. Davin, J. Biol. Chem. **267**, 21235 (1992).
18. E. Zhang *et al.*, *Nat. Med.* **16**, 1152 (2010).
19. A. N. Dodd *et al.*, *Science* **309**, 630 (2005).
20. E. M. Dioum *et al.*, *Science* **298**, 2385 (2002).
21. P. McNamara *et al.*, *Cell* **105**, 877 (2001).
22. A. Y. So, T. U. Bernal, M. L. Pillsbury, K. R. Yamamoto, B. J. Feldman, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 17582 (2009).
23. B. Kornmann, O. Schaad, H. Bujard, J. S. Takahashi, U. Schibler, *PLoS Biol.* **5**, e34 (2007).
24. A. Balsalobre *et al.*, *Science* **289**, 2344 (2000).
25. S. Kiehlisch, G. Eichele, H. Oster, *J. Clin. Invest.* **120**, 2600 (2010).
26. G. M. Sutton *et al.*, *J. Neurosci.* **28**, 12946 (2008).
27. E. D. Buhr, S. H. Yoo, J. S. Takahashi, *Science* **330**, 379 (2010).
28. H. Duez *et al.*, *Gastroenterology* **135**, 689 (2008).
29. F. W. Turek *et al.*, *Science* **308**, 1043 (2005).
30. J. E. Baggs *et al.*, *PLoS Biol.* **7**, e52 (2009).
31. M. Kawai *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 10508 (2010).
32. C. B. Green *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 9888 (2007).
33. M. Kawai, A. M. Delany, C. B. Green, M. L. Adamo, C. J. Rosen, *Endocrinology* **151**, 4861 (2010).
34. T. Kudo, T. Tamagawa, M. Kawashima, N. Mito, S. Shibata, *J. Biol. Rhythms* **22**, 312 (2007).
35. G. Cretenet, M. Le Clech, F. Gachon, *Cell Metab.* **11**, 47 (2010).
36. M. E. Hughes *et al.*, *PLoS Genet.* **5**, e1000442 (2009).
37. X. Wu *et al.*, *Obesity (Silver Spring)* **15**, 2560 (2007).
38. X. Wu *et al.*, *Int. J. Obesity (London)* **33**, 971 (2009).
39. D. J. Durgan, M. E. Young, *Circ. Res.* **106**, 647 (2010).
40. C. B. Anea *et al.*, *Circulation* **119**, 1510 (2009).
41. A. M. Curtis *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 3450 (2007).
42. N. Allaman-Pillet *et al.*, *Mol. Cell. Endocrinol.* **226**, 59 (2004).
43. D. E. Ayala *et al.*, *Chronobiol. Int.* **26**, 1189 (2009).
44. E. J. Westgate *et al.*, *Circulation* **117**, 2087 (2008).
45. J. A. Schoenhard *et al.*, *J. Mol. Cell. Cardiol.* **35**, 473 (2003).
46. K. S. Polonsky *et al.*, *N. Engl. J. Med.* **318**, 1231 (1988).
47. G. Boden, X. Chen, M. Polansky, *Diabetes* **48**, 2182 (1999).
48. A. Kalsbeek *et al.*, *PLoS ONE* **3**, e3194 (2008).
49. E. Peschke, D. Peschke, *Diabetologia* **41**, 1085 (1998).
50. B. Marcheva *et al.*, *Nature* **466**, 627 (2010).
51. K. A. Lamia, K. F. Storch, C. J. Weitz, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 15172 (2008).
52. J. Dupuis *et al.*, *Nat. Genet.* **42**, 105 (2010).
53. H. Mulder, C. L. Nagorny, V. Lysenko, L. Groop, *Diabetologia* **52**, 1240 (2009).
54. E. Tasali, R. Leproult, D. A. Ehrmann, E. Van Cauter, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 1044 (2008).
55. C. B. Saper, T. E. Scammell, J. Lu, *Nature* **437**, 1257 (2005).
56. A. Adamantidis, L. de Lecea, *Trends Endocrinol. Metab.* **19**, 362 (2008).
57. H. Funato *et al.*, *Cell Metab.* **9**, 64 (2009).
58. S. Taheri, J. M. Zeitzer, E. Mignot, *Annu. Rev. Neurosci.* **25**, 283 (2002).
59. A. D. Laposky, M. A. Bradley, D. L. Williams, J. Bass, F. W. Turek, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **295**, R2059 (2008).
60. F. A. Scheer, M. F. Hilton, C. S. Mantzoros, S. A. Shea, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 4453 (2009).
61. A. Kohsaka *et al.*, *Cell Metab.* **6**, 414 (2007).
62. K. A. Lamia *et al.*, *Science* **326**, 437 (2009).
63. G. Asher *et al.*, *Cell* **134**, 317 (2008).
64. Y. Nakahata *et al.*, *Cell* **134**, 329 (2008).
65. K. M. Ramsey *et al.*, *Science* **324**, 651 (2009).
66. Y. Nakahata, S. Sahar, G. Astarita, M. Kaluzova, P. Sassone-Corsi, *Science* **324**, 654 (2009).
67. J. T. Rodgers, P. Puigserver, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 12861 (2007).
68. G. Asher *et al.*, *Cell* **142**, 943 (2010).

We thank R. Allada, F. Turek, B. Chung and K.-M. Ramsey for helpful suggestions and B. Marcheva for figures. This work was supported by NIH (P01 AG011412 and R01HL097817), Chicago Biomedical Consortium Searle Funds, Islet Biology Core of the University of Chicago Diabetes Research and Training Center, American Diabetes Association, and Juvenile Diabetes Research Foundation to J.B., and NIH P50 MH074924 and R01 MH078024 to J.S.T. J.S.T. is an investigator in the Howard Hughes Medical Institute. J.S.T. is a cofounder of ReSet Therapeutics, Inc., and J.S.T. and J.B. are members of its scientific advisory board. J.B. is also an advisor and receives support from Amylin Pharmaceuticals. Patents have been applied for related to J.B.'s work on therapeutic applications of NAD and melatonin.



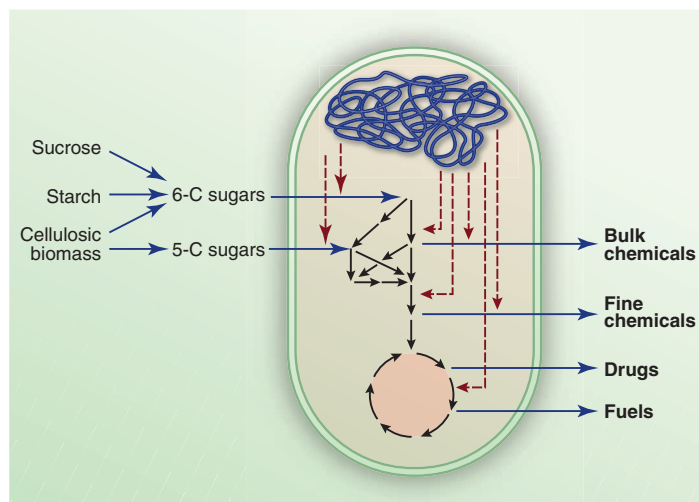
## REVIEW

# Manufacturing Molecules Through Metabolic Engineering

Jay D. Keasling<sup>1,2,3</sup>

Metabolic engineering has the potential to produce from simple, readily available, inexpensive starting materials a large number of chemicals that are currently derived from nonrenewable resources or limited natural resources. Microbial production of natural products has been achieved by transferring product-specific enzymes or entire metabolic pathways from rare or genetically intractable organisms to those that can be readily engineered, and production of unnatural specialty chemicals, bulk chemicals, and fuels has been enabled by combining enzymes or pathways from different hosts into a single microorganism and by engineering enzymes to have new function. Whereas existing production routes use well-known, safe, industrial microorganisms, future production schemes may include designer cells that are tailor-made for the desired chemical and production process. In any future, metabolic engineering will soon rival and potentially eclipse synthetic organic chemistry.

The term “metabolic engineering” was coined in the late 1980s–early 1990s (1). Since that time, the range of chemicals that can be produced has expanded substantially, in part due to notable advances in fields adjacent to metabolic engineering: DNA sequencing efforts have revealed new metabolic reactions and variants of enzymes from many different organisms; extensive databases of gene expression, metabolic reactions, and enzyme structures allow one to query for desired reactions and design or evolve novel enzymes for reactions that do not exist; new genetic tools enable more precise control over metabolic pathways; new analytical tools enable the metabolic engineer to track RNA, protein, and metabolites in a cell to identify pathway bottlenecks; and detailed models of biology aid in the design of enzymes and metabolic pathways. Yet even with these substantial developments, microbial catalysts are not as malleable as those in synthetic organic chemistry, and metabolic engineers must weigh many trade-offs in the development of microbial catalysts: (i) cost and availability of starting materials (e.g., carbon substrates); (ii) metabolic route and corresponding genes encoding the enzymes in the pathway to produce the desired product; (iii) most appropriate microbial host; (iv) robust and responsive genetic control system for the desired pathways and chosen host; (v) methods for debugging and debottlenecking the constructed



**Fig. 1.** Conversion of sugars to chemicals by means of microbial catalysts.

pathway; and (vi) ways to maximize yields, titers, and productivities (Fig. 1). Unfortunately, these design decisions cannot be made independently of each other: Genes cannot be expressed, nor will the resulting enzymes function, in every host; products or metabolic intermediates may be toxic to one host but not another host; different hosts have different levels of sophistication of genetic tools available; and processing conditions (e.g., growth, production, product separation and purification) are not compatible with all hosts. Even with these many challenges, metabolic engineering has been successful for many applications, and with continued developments more applications will be possible.

## Starting Materials, Products, and Metabolic Routes

One area where metabolic engineering has a sizable advantage over synthetic organic chemistry is in the production of natural products,

particularly active pharmaceutical ingredients (APIs), some of which are too complex to be chemically synthesized and yet have a value that justifies the cost of developing a genetically engineered microorganism. The cost of starting materials is generally a small fraction of their cost, and relatively little starting material is necessary so availability is not an issue. Most APIs fall into three classes of natural products, and many of the biosynthetic pathways for their precursors have been reconstituted in heterologous hosts.

Alkaloids are nitrogen-containing, low molecular weight compounds found primarily in and derived from plants and widely used as drugs. Two recent studies conclude that the large group of benzyl isoquinoline alkaloids (BIAs) will one day be producible in *Escherichia coli* and *Saccharomyces cerevisiae* (2). Unfortunately, the BIAs are only one of four major alkaloid groups, all of which are produced through different pathways. As the metabolic pathways for other alkaloids are discovered in their natural producers, many more of these valuable molecules could be produced microbially.

Polyketides and nonribosomal peptides (NRPs) have found broad use as APIs, veterinary agents, and agrochemicals. Naturally occurring polyketides and NRPs are produced by a number of bacteria and fungi using large, modular enzymes. Their titers and yields in the native producers have been improved through traditional strain engineering and advanced metabolic engineering. More recently, some of the most valuable molecules have been produced with engineered industrial hosts (3). Recombination of various synthase modules allows one to produce a nearly infinite range of chemicals

(4, 5), opening up the possibility that they may one day be used to produce fine and bulk chemicals.

Isoprenoids have found use as fragrances and essential oils, nutraceuticals, and pharmaceuticals. Many isoprenoids have been produced microbially, including carotenoids and various plant-derived terpenes (6–8), taking advantage of terpene synthases to form the most complicated part of the molecules and hydroxylases to introduce hydroxyl group that can be subsequently functionalized chemically or biologically (7, 9). Isoprenoids are one of the few classes of natural products where there are alternative precursor production pathways. An example of using metabolic engineering and synthetic chemistry together to produce an API is the semisynthesis of the antimalarial drug artemisinin with *S. cerevisiae* engineered to produce artemisinic acid, the most complex part of the molecule, and synthetic chemistry to produce artemisinin from the microbially sourced

<sup>1</sup>Joint BioEnergy Institute, 5885 Hollis Street, Emeryville, CA 94608, USA. <sup>2</sup>Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA. <sup>3</sup>Synthetic Biology Engineering Research Center, Department of Chemical and Biomolecular Engineering, University of California, Berkeley, CA 94720, USA. E-mail: keasling@berkeley.edu

artemisinic acid (6, 7, 9). Beyond producing natural products, laboratory evolution or rational engineering of terpene cyclases, terpene hydroxylases, and a host of other terpene-functionalizing enzymes (8, 10–12) and combinatorial expression of these evolved enzymes in a heterologous host will enable the production of unnatural terpenes, some of which might be more effective than the natural product for the treatment of human disease.

Although individual metabolic pathways have been developed to produce natural products derived from a single pathway, there is an opportunity to synthesize multisubstituent APIs (e.g., Taxol) or other molecules from the products of multiple biosynthetic pathways. This will require simultaneous expression of multiple precursor pathways in a single microorganism, as well as “ligases” that can assemble multiple substituents together into a single molecule. The benefit would be the synthesis of complicated molecules that might not otherwise be produced.

Although not as valuable as pharmaceuticals, many fine chemicals have been produced economically from natural and engineered microorganisms, including amino acids, organic acids, vitamins, flavors, fragrances, and nutraceuticals. For fine chemicals, profit margins are generally much lower than for APIs and may be affected by substrate availability and cost. Some of these molecules are sufficiently complicated that they cannot be produced economically by any route other than biological production, whereas others have chemical routes. For some important products (fragrances, flavors, amino acids), heterologous hosts have been engineered to enhance their production. Yet we have barely begun to investigate what will be possible to produce.

In contrast, bulk chemicals such as solvents and polymer precursors are rarely produced from microorganisms, because they can be produced inexpensively from petroleum by chemical catalysis. Due to fluctuations in petroleum prices and recognition of dwindling reserves, trade imbalances, and political considerations, it is now possible to consider production of these inexpensive chemicals from low-cost starting materials such as starch, sucrose, or cellulosic biomass (e.g., agricultural and forest waste, dedicated energy crops, etc.) with a microbial catalyst. For example, 1,3-propanediol (1,3-PDE), a useful intermediate in the synthesis of polyurethanes and polyesters, is now being produced from glucose by *E. coli* engineered with genes from *Klebsiella pneumoniae* and *S. cerevisiae* (13). There is an opportunity to produce many other bulk chemicals (e.g., polymer precursors) by using metabolically engineered cells, but the key will be to produce the exact molecule needed for existing products rather than something “similar but green” that will require extensive product testing before it can be used.

By far the highest-volume (and lowest-margin) application for engineered metabolism

is the production of transportation fuels. For many of the same reasons that it is desirable to produce petroleum-derived chemicals using biological systems, it is desirable to produce transportation fuels from readily available, inexpensive, renewable sources of carbon. There is a long history of using microorganisms to produce alcohols, primarily ethanol and butanol. Although much of the work on these alcohols was done by traditional strain mutagenesis and selection, more recent work has focused on engineering yeasts and bacteria to produce ethanol or butanol from a variety of sugars while eliminating routes to side products and improving the tolerance of the host to the alcohol (14). Larger, branched-chain alcohols can be produced by way of the Ehrlich pathway. By incorporating broad substrate-range 2-keto acid decarboxylases and alcohol dehydrogenases, several microbes have now been engineered to produce these fuels (15, 16). These alcohols are generally considered better fuels than ethanol and butanol and can also be used to produce a variety of commodity chemicals.

Recent advances in metabolic pathway and protein engineering have made it possible to engineer microorganisms to produce hydrocarbons with properties similar or identical to those of petroleum-derived fuels and thus compatible with our existing transportation infrastructure. Linear hydrocarbons (alkanes, alkenes, and esters) typical of diesel and jet fuel can be produced by way of the fatty acid biosynthetic pathway (17–19). For diesel in cold weather and jet fuel at high altitudes, branches in the chain are beneficial—regularly branched and cyclic hydrocarbons of different sizes with diverse structural and chemical properties can be produced via the isoprenoid biosynthetic pathway (20, 21). Both the fatty acid-derived and the isoprenoid-derived fuels diffuse (or are pumped) out of the engineered cells and phase separate in the fermentation, making purification simple and reducing fuel cost.

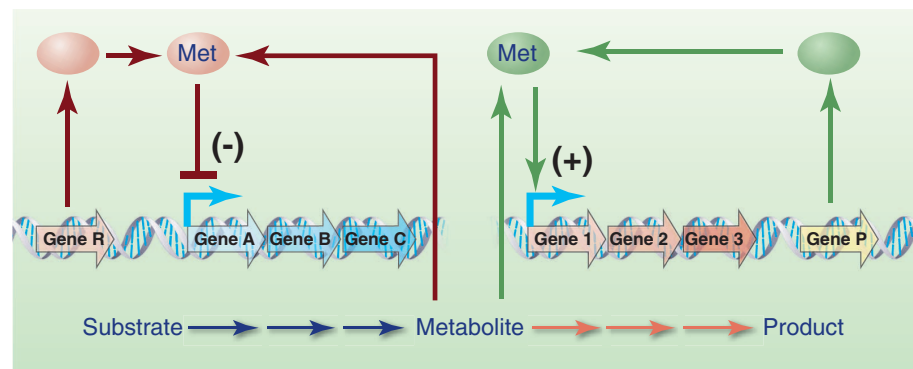
Although the pathways described above produce a wide range of fuel-like molecules, there

are many other molecules that one might want to produce, such as short, highly branched hydrocarbons (e.g., 2,2,4-trimethyl pentane or isooctane) that would be excellent substitutes for petroleum-derived gasoline. Additionally, most petroleum fuels are mixtures of large numbers of components that together create the many important properties of the fuels. It should be possible to engineer single microbes or microbial consortia to produce a mixture of fuels from one of the biosynthetic pathways or from multiple biosynthetic pathways. Indeed, some enzymes produce mixtures of products from a single precursor—maybe these enzymes could be tuned to produce a fuel mixture ideal for a particular engine type or climate.

To make these new fuels economically viable, we must tap into inexpensive carbon sources (namely, sugars from cellulosic biomass). Given the variety of sugars in cellulosic biomass, the fuel producer must be able to consume both five- and six-carbon sugars. Because many yeasts do not consume five-carbon sugars, recent developments in engineering yeast to catabolize these sugars will make production of these fuels more economically viable (22). Engineering fuel-producing microorganisms to secrete cellulases and hemicellulases to depolymerize these sugar polymers into sugars before uptake and conversion into fuels has the potential to substantially reduce the cost of producing the fuel.

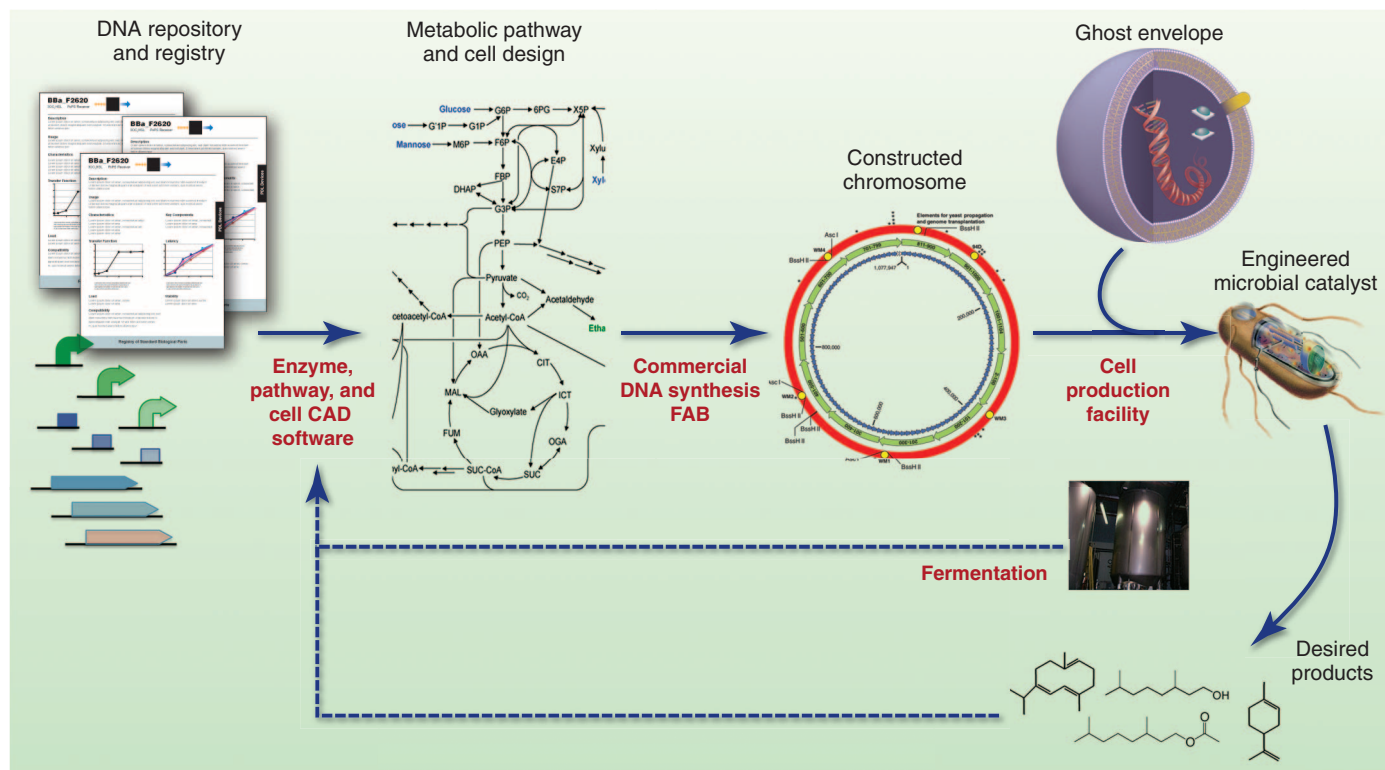
## Hosts and Expression Systems

From the applications cited above, it should be evident that the product, starting materials, and production process all affect host choice. Some of the most important qualities one must consider when choosing a host are whether the desired metabolic pathway exists or can be reconstituted in that host; if the host can survive (and thrive) under the desired process conditions (e.g., ambient versus extremes of temperature, pH, ionic strength, etc.); if the host is genetically stable (both with the introduced pathway and not susceptible to phage attack); and if good genetic tools are available to



**Fig. 2.** Use of synthetic regulators to modulate metabolic pathways that have a toxic intermediate. Regulatory proteins or RNAs bind the toxic metabolite and down-regulate the biosynthetic pathway and up-regulate the consumption pathway.





**Fig. 3.** The future of engineered biocatalysts. Pathways, enzymes, and genetic controls are designed from characteristics of parts (enzymes, promoters, etc.) by means of pathway and enzyme CAD software. The chromosomes encoding

those elements are synthesized at a FAB and incorporated into a ghost envelope to obtain the new catalyst. The design of the engineered catalyst is influenced by the desired product and the production process.

manipulate the host. Widely used, heterologous hosts include *E. coli*, *S. cerevisiae*, *Bacillus subtilis*, and *Streptomyces coelicolor*, to name a few. Although *E. coli* and *S. cerevisiae* excel in the genetic tools available, *E. coli* has the disadvantage of being susceptible to phage attack. And while *B. subtilis* and *S. coelicolor* have the ability to easily express polyketide synthases, they have fewer genetic tools available than either *S. cerevisiae* or *E. coli*. Although minimal, bacterial hosts may have scientific interest (23), minimal hosts that require addition of many nutrients or that cannot cope with stresses in processing will probably not find a niche in industrial chemical or fuel production where cost is critical. Thus, it is essential to have genetic tools for existing industrial hosts that can grow on simple, inexpensive carbon sources and salts or on an inexpensive, undefined medium with minimal additions (24, 25).

The key issue necessitating good genetic tools is the introduction of foreign genes encoding the metabolic pathway and control over their expression to maximize yields and titers. The genes encoding the transformational enzymes in metabolically engineered cells do not need to be highly expressed, but must be produced in catalytic amounts sufficient to adequately transform the metabolic intermediates into the desired products at a sufficient rate. Expression of the desired genes at too high a level will rob the cell of metabolites

that might otherwise be used to produce the desired molecule of interest, particularly important for production of low-margin chemicals, while underexpressed genes will create pathway bottlenecks. Furthermore, because intermediates of a foreign metabolic pathway can be toxic to the heterologous host (6), which results in decreased production of the desired final compound, it is essential that the relative levels of the enzymes be coordinated.

Central to any genetic manipulation is the vector used to carry and/or harbor the transforming DNA in the host. Important features of the cloning vector include segregational stability, minimal and consistent copy number in all cells of a culture, and the ability to replicate and express large sequences of DNA. There is growing recognition that one or only a few copies of a gene are needed, particularly for metabolic engineering applications. With the ability to vary promoter (26) and ribosome binding strength (27), as well as the stabilities of the mRNA (28) and the resulting protein produced from it, there are many factors other than copy number that can be manipulated to alter enzyme production.

Promoters play an essential role in controlling biosynthetic pathways. Inducible promoters are one of the easiest and most effective ways to regulate gene expression, but it is essential that the promoter be induced consistently in all cells

of a culture (29). Constitutive promoters (26) and promoters that respond to a change in growth condition or to an important intermediary metabolite (30) allow for inexpensive, inducer-free gene expression, which is particularly important where cost is an issue (Fig. 2). Although there are many inducible promoters for bacteria, the small number of inducible promoters for yeast and other potential industrial hosts makes regulation of metabolic pathways in those organisms more challenging than in bacteria.

Because production of complicated molecules often requires several enzymes, it is desirable to coordinate expression of the genes encoding these enzymes to prevent accumulation of toxic intermediates and bottlenecks in biosynthetic pathways. There are many ways to coordinate expression of multiple genes, such as using a non-native RNA polymerase or transcription factor to induce multiple promoters (31); grouping multiple, related genes into operons; varying the ribosome binding strength for the enzymes encoded in the operon (27); or controlling segmental mRNA stability of each coding region to regulate the amount of each enzyme produced (32). One of the limitations to expressing multiple genes in yeast is the lack of internal ribosomal entry sequences (IRESs) that are available for higher eukaryotes. The development of yeast IRESs would allow one to express genes encoding metabolic

pathways without the need for a promoter for each gene.

## Debottlenecking, Debugging, and Process Optimizing

Even with a kit full of tools, building a biosynthetic pathway is made difficult without accurate blueprints. In almost all areas of engineering, there are models and simulation tools that allow one to predict which components to assemble to obtain a larger system with a desired function or characteristic. Similar biological design tools are in their infancy. However, metabolic models that incorporate cell composition and gene regulation have become relatively predictive and may one day be used to design metabolic pathways and predict the level of gene expression needed to achieve a particular flux through a reaction or pathway (33).

Regardless of how sophisticated the design tools and how good the blueprint, there will always be “bugs” in the engineered system. Analogous to software debuggers that allow one to find and fix errors in computer code, the development of similar tools for biological debugging would reduce development times for optimizing engineered cells. For the development of microbial chemical factories, functional genomics can serve in the role of debugging routines (34), because imbalances in a metabolic pathway often elicit a stress response in central metabolism (due to protein overproduction or accumulation of toxic intermediates or end products) (6, 35). Information from one or more of these techniques can be used to diagnose the problem and modify expression of genes in the metabolic pathway or in the host to improve titer and/or productivity.

Many desirable chemicals will be toxic to the producer, particularly at the high titers needed for industrial-scale production. Taking advantage of the cell’s native stress response pathways can be an effective way to alleviate part or all of the toxicity (36). Even better, transporters could be used to pump the desired product outside the cell, reducing intracellular toxicity and purifying the product from the thousands of contaminating intracellular metabolites (37).

## Designer Cells for Designer Chemicals

One can envision a future when a microorganism is tailor-made for production of a specific chemical from a specific starting material, much like chemical engineers build refineries and other chemical factories from unit operations (Fig. 3). The chemical and physical characteristics of the product and starting materials would be considered in the design of the organism to minimize both production and purification costs (e.g., operating the engineered cell at the boiling point of volatile, toxic products to drive production and reduce product toxicity). The cell envelope would be designed to be resistant to the specific desired chemical, and the cell wall would be designed to make the organism tolerant to indus-

trial processing conditions. Specific, engineered transporters would be incorporated into the membrane to pump the desired product out of the cell and keep it out and to import the desired starting material. The biosynthetic pathway would be constructed from a parts registry containing all known enzymes by means of retrosynthesis software (38), and done so to maximize yield and minimize the time required to grow the organism and produce the desired chemical from the desired starting material. In the event that an enzyme does not exist for a particular reaction or set of reactions, one would use computer-aided design (CAD) software to design the desired enzyme (39).

Once the cell has been designed in the computer, the genetic control system would be designed to control expression of all the genes at the correct time and at the appropriate levels. Redundancies in the genetic control system would be engineered to ensure that design parameters are maintained regardless of the transient changes the cell encounters during the production process. Simulations and scenario planning would test various designs, including genetic control system failure. Safety for the plant operators and the environment would be an essential design criterion. When the genetic controls were fully designed and tested, the chromosome(s) would be designed and constructed. Gene location, modularity, and ease of construction are but a few of the important considerations in designing the chromosome. The chromosome would be ordered from a commercial DNA manufacturer. Depending on the state of the technology at the time, the chromosome would arrive in pieces and be assembled in the constructed envelope or would be completely assembled at the factory and sent to another location to be introduced into the host cell. One can even envision a day when cell manufacturing is done by different companies, each specializing in certain aspects of the synthesis—one company constructs the chromosome, one company builds the membrane and cell wall (the “bag”), one company fills the bag with the basic molecules needed to boot up the cell.

Until this future arrives, manufacturing of molecules will be done with well-known, safe, industrial microorganisms that have tractable genetic systems. Continued development of tools for existing, safe, industrial hosts, cloning and expressing genes encoding precursor production pathways, and the creation of novel enzymes that catalyze unnatural reactions will be necessary to expand the range of products that can be produced from biological systems. When more of these tools are available, metabolic engineering should be just as powerful as synthetic chemistry, and together the two disciplines can greatly expand the number of products available from renewable resources.

## References and Notes

1. J. E. Bailey, *Science* **252**, 1668 (1991).
2. K. M. Hawkins, C. D. Smolke, *Nat. Chem. Biol.* **4**, 564 (2008).

3. B. A. Pfeifer, S. J. Admiraal, H. Gramajo, D. E. Cane, C. Khosla, *Science* **291**, 1790 (2001).
4. H. G. Menzella et al., *Nat. Biotechnol.* **23**, 1171 (2005).
5. V. Siewers, R. San-Bento, J. Nielsen, *Biotechnol. Bioeng.* **106**, 841 (2010).
6. V. J. J. Martin, D. J. Pitera, S. T. Withers, J. D. Newman, J. D. Keasling, *Nat. Biotechnol.* **21**, 796 (2003).
7. D. K. Ro et al., *Nature* **440**, 940 (2006).
8. E. Leonard et al., *Proc. Natl. Acad. Sci. U.S.A.* **107**, 13654 (2010).
9. M. C. Chang, R. A. Eachus, W. Trieu, D. K. Ro, J. D. Keasling, *Nat. Chem. Biol.* **3**, 274 (2007).
10. Y. Yoshikuni, T. E. Ferrin, J. D. Keasling, *Nature* **440**, 1078 (2006).
11. C. Schmidt-Dannert, D. Umeno, F. H. Arnold, *Nat. Biotechnol.* **18**, 750 (2000).
12. J. A. Dietrich et al., *ACS Chem. Biol.* **4**, 261 (2009).
13. C. E. Nakamura, G. M. Whited, *Curr. Opin. Biotechnol.* **14**, 454 (2003).
14. E. J. Steen et al., *Microb. Cell Fact.* **7**, 36 (2008).
15. G. K. Donaldson, A. C. Eliot, D. Flint, L. A. Maggio-Hall, V. Nagarajan, U.S. Patent 20070092957 (2007).
16. S. Atsumi, T. Hanai, J. C. Liao, *Nature* **451**, 86 (2008).
17. E. J. Steen et al., *Nature* **463**, 559 (2010).
18. H. R. Beller, E.-B. Goh, J. D. Keasling, *Appl. Environ. Microbiol.* **76**, 1212 (2010).
19. A. Schirmer, M. A. Rude, X. Li, E. Popova, S. B. del Cardayre, *Science* **329**, 559 (2010).
20. S. T. Withers, S. S. Gottlieb, B. Lieu, J. D. Newman, J. D. Keasling, *Appl. Environ. Microbiol.* **73**, 6277 (2007).
21. N. S. Renninger, D. J. McPhee, World Patent 200804555 (2008).
22. H. W. Wisselink, M. J. Toirkens, Q. Wu, J. T. Pronk, A. J. van Maris, *Appl. Environ. Microbiol.* **75**, 907 (2009).
23. D. G. Gibson et al., *Science* **329**, 52 (2010).
24. H. H. Wang et al., *Nature* **460**, 894 (2009).
25. G. Pósfai et al., *Science* **312**, 1044 (2006).
26. P. R. Jensen, K. Hammer, *Appl. Environ. Microbiol.* **64**, 82 (1998).
27. H. M. Salis, E. A. Mirsky, C. A. Voigt, *Nat. Biotechnol.* **27**, 946 (2009).
28. C. D. Smolke, T. A. Carrier, J. D. Keasling, *Appl. Environ. Microbiol.* **66**, 5399 (2000).
29. A. Khlebnikov, K. A. Datsenko, T. Skaug, B. L. Wanner, J. D. Keasling, *Microbiology* **147**, 3241 (2001).
30. W. R. Farmer, J. C. Liao, *Nat. Biotechnol.* **18**, 533 (2000).
31. H. Alper, G. Stephanopoulos, *Metab. Eng.* **9**, 258 (2007).
32. B. F. Pfeleger, D. J. Pitera, C. D. Smolke, J. D. Keasling, *Nat. Biotechnol.* **24**, 1027 (2006).
33. J. S. Edwards, R. U. Ibarra, B. O. Palsson, *Nat. Biotechnol.* **19**, 125 (2001).
34. J. H. Park, K. H. Lee, T. Y. Kim, S. Y. Lee, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 7797 (2007).
35. L. Kizer, D. J. Pitera, B. F. Pfeleger, J. D. Keasling, *Appl. Environ. Microbiol.* **74**, 3229 (2008).
36. H. Alper, J. Moxley, E. Nevoigt, G. R. Fink, G. Stephanopoulos, *Science* **314**, 1565 (2006).
37. M. J. Dunlop, J. D. Keasling, A. Mukhopadhyay, *Syst. Synth. Biol.* **4**, 95 (2010).
38. K. L. Prather, C. H. Martin, *Curr. Opin. Biotechnol.* **19**, 468 (2008).
39. J. B. Siegel et al., *Science* **329**, 309 (2010).
40. This work was supported in part by the Synthetic Biology Engineering Research Center, which is funded by National Science Foundation Award No. 0540879 and by the Joint BioEnergy Institute, which is funded by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, through contract DE-AC02-05CH11231. Competing financial interests: The author is a founder of and owns equity in Amyris and LS9.

10.1126/science.1193990



# How Learning to Read Changes the Cortical Networks for Vision and Language

Stanislas Dehaene,<sup>1,2,3,4,\*</sup> Felipe Pegado,<sup>1,2,3</sup> Lucia W. Braga,<sup>5</sup> Paulo Ventura,<sup>6</sup> Gilberto Nunes Filho,<sup>5</sup> Antoinette Jobert,<sup>1,2,3</sup> Ghislaine Dehaene-Lambertz,<sup>1,2,3</sup> Régine Kolinsky,<sup>7,8</sup> José Morais,<sup>7</sup> Laurent Cohen<sup>9,10,11</sup>

Does literacy improve brain function? Does it also entail losses? Using functional magnetic resonance imaging, we measured brain responses to spoken and written language, visual faces, houses, tools, and checkers in adults of variable literacy (10 were illiterate, 22 became literate as adults, and 31 were literate in childhood). As literacy enhanced the left fusiform activation evoked by writing, it induced a small competition with faces at this location, but also broadly enhanced visual responses in fusiform and occipital cortex, extending to area V1. Literacy also enhanced phonological activation to speech in the planum temporale and afforded a top-down activation of orthography from spoken inputs. Most changes occurred even when literacy was acquired in adulthood, emphasizing that both childhood and adult education can profoundly refine cortical organization.

Practically all adult neuroimaging experiments are performed in highly educated college students. The observed brain architecture therefore reflects the influence of culture and education over and above spontaneous brain development (1, 2). Indeed, the acquisition of reading, a major event in children's lives, is now recognized as capable of changing both brain anatomy (3, 4) and brain activation (5–9). In the auditory modality, literacy leads to phonemic awareness, the ability to manipulate the smallest units of spoken language [i.e., phonemes (10)], and alters online speech processing (11–14). At the visual level, developmental neuroimaging studies in normal and dyslexic children show that, with reading acquisition, a specific brain site in left occipito-temporal cortex, which has been termed “visual word form area” (VWFA), starts to respond to orthographic stimuli in the learned script (15–19).

These observations leave many important questions unanswered. First, does literacy primarily lead to cooperative or to competitive effects

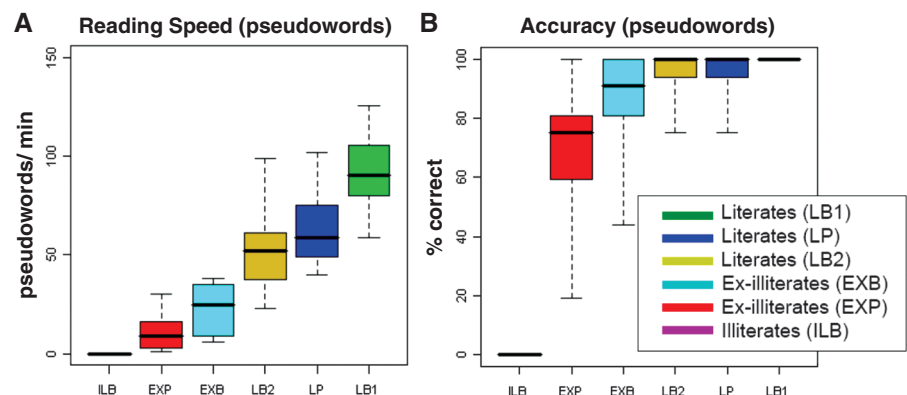
on cortical processing? Two theoretical positions can be contrasted. The first view, derived from animal studies of environmental enrichment and sensory plasticity, emphasizes that perceptual learning entails beneficial modifications of cortical maps, including sharpened receptive fields and neuronal tuning curves correlated with behavioral improvements (20–22). Without denying these positive effects, the second view emphasizes that reading is a cultural invention too recent to involve dedicated genetic or developmental mechanisms. Thus, during education, reading processes must invade and “recycle” cortical space devoted to evolutionary older functions, opening the possibility that these functions suffer as reading expertise sets in (2, 23). Much like expertise for nonface stimuli induces a reduction in face responses (24–26), reading, which recruits an identical cortical site in all cultures (27), might entail a reorganization of nearby responses to faces,

houses, and objects. We thus sought to understand which of these stimuli are processed in the VWFA area before reading and how their cortical representation, which gets refined during the school years (28), is affected by literacy.

A second issue is that, at present, most functional imaging studies of illiteracy only contrasted schooled versus unschooled adults. Because these studies did not include “ex-illiterate” adults who did not attend school but learned to read during adulthood, they confounded the effects of schooling and literacy. The only important exception (4) focused solely on how brain anatomy is changed by literacy. In this study, we separated the functional effects of schooling and literacy by comparing illiterates, ex-illiterates, and adults schooled in childhood.

**Populations studied and verification of literacy level.** We scanned a total of 63 Portuguese and Brazilian participants. Our sample included 32 unschooled adults (10 illiterates and 22 ex-illiterates with variable reading skills), and 31 schooled and literate adults. The latter group included 11 literate subjects matched to the illiterates in socioeconomic status (SES) (29). Reading skills were verified through behavioral tasks of letter identification, word and pseudo-word reading (with or without speed pressure), and sentence reading (Fig. 1, fig. S1, and table S1). All tests revealed the same ordering of literacy, from Brazilian illiterates (ILB) to Portuguese ex-illiterates (EXP), Brazilian ex-illiterates (EXB), low-SES Brazilian literates (LB2), Portuguese literates (LP), and Brazilian literates (LB1). We therefore relied on whole-brain linear regressions with reading performance (number of stimuli read per minute) across all groups to identify the brain regions influenced by literacy. Once identified, each brain site was submitted to restricted comparisons of subgroups to evaluate the effects of schooling and literacy with maximal sensitivity (29).

We used three types of whole-brain functional magnetic resonance (fMRI) runs: a “localizer” with horizontal and vertical checkerboards, written and



**Fig. 1.** The six groups of participants and their reading skills. Box plots show (A) the speed and (B) accuracy in reading a list of pseudowords (central horizontal line, median; box, 25th and 75th percentiles; whiskers, minimum and maximum). Additional data on word and sentence reading are provided in fig. S1.

<sup>1</sup>INSERM, Cognitive Neuroimaging Unit, Gif sur Yvette 91191, France. <sup>2</sup>Commissariat à l’Énergie Atomique, Direction des Sciences du Vivant, I2BM, Neurospin center, Gif sur Yvette 91191, France. <sup>3</sup>University Paris-Sud 11, 91405 Orsay, France. <sup>4</sup>Collège de France, 11 Place Marcelin Berthelot, 75005 Paris, France. <sup>5</sup>SARAH Network—International Center for Neurosciences and Rehabilitation, QL 13, Lago Norte, 71.535-005 Brasília, DF Brazil. <sup>6</sup>Faculty of Psychology, University of Lisbon, 1600-214 Lisbon, Portugal. <sup>7</sup>Faculty of Psychology, Université Libre de Bruxelles (ULB), 1050 Brussels, Belgium. <sup>8</sup>Fonds de la Recherche Scientifique (FNRS), 1000 Brussels, Belgium. <sup>9</sup>Université Pierre et Marie Curie-Paris 6, Faculté de Médecine Pitié-Salpêtrière, 75013 Paris, France. <sup>10</sup>Assistance Publique-Hôpitaux de Paris, Groupe hospitalier Pitié-Salpêtrière, Department of Neurology, 75651 Paris, France. <sup>11</sup>INSERM, Centre de Recherches de l’Institut du cerveau et de la moelle épinière, UMRs 975, Paris, France.

\*To whom correspondence should be addressed. E-mail: stanislas.dehaene@gmail.com

spoken sentences, motor commands, and calculation problems (fig. S2); three visual runs evaluating cortical responses to faces, houses, tools, letter strings, false fonts, and moving checkerboards, while the participant focused on detecting a target star (fig. S3); and four auditory lexical-decision runs with spoken stimuli. To verify compliance, during the localizer, participants either heard or saw short verbal instructions to perform simple calculations or to click the left or right button. With spoken instructions, we observed classical regions for calculation and hand movements, without modulation by literacy, indicating similar comprehension and compliance in all groups. With written instructions, however, activation at the same locations was strongly modulated by reading performance, varying from zero activation in illiterates to a level equivalent to spoken instructions in literates (see fig. S4). While unsurprising, these results validate our group definitions and literacy measure and establish that, with spoken materials, all groups followed instructions quite well. Thus, any subsequent differences cannot be attributed to lower attention or comprehension in illiterates.

**Responses to written sentences enhanced by literacy.** We first examined, in the localizer run, which regions were modulated by reading performance during the viewing of simple sentences consisting of serially presented written words (Fig. 2 and fig. S7). A massive effect was seen in the left ventral occipito-temporal cortex, at classical VWFA coordinates ( $-40, -50, -14$ ,  $Z$  score = 6.86); with posterior subpeaks ( $-46, -70, -18$ ,  $Z = 5.39$ ;  $-32, -80, -8$ ,  $Z = 3.96$ ); and at a right occipital site ( $22, -86, -10$ ,  $Z = 5.17$ ). These regions were strictly visual, as attested by their lack of activation to spoken sentences. Modulation by reading performance was also seen in a vast left-hemisphere language network, which was also activated by spoken language in all groups: left posterior, middle, and anterior superior temporal sulcus (STS;  $-50, -44, 6$ ,  $Z = 7.10$ ;  $-54, -12, -12$ ,  $Z = 5.42$ ); left temporal pole ( $-50, 12, -24$ ,  $Z = 4.13$ ); left and right premotor cortex ( $-46, -2, 52$ ,  $Z = 8.50$ ;  $46, 4, 40$ ,  $Z = 5.48$ ); left inferior frontal gyrus ( $-54, 26, -6$ ,  $Z = 5.76$ ); and left supplementary motor area ( $-4, 2, 62$ ,  $Z = 6.33$ ). A significant left-hemispheric asymmetry of this effect was observed in all areas except temporal pole and occipital cortex. Direct comparison of spoken versus written stimuli showed that, in the literate participants, frontal regions became equally activated by spoken versus written language, whereas temporal areas overlapped but still showed a significant difference favoring spoken language (fig. S8).

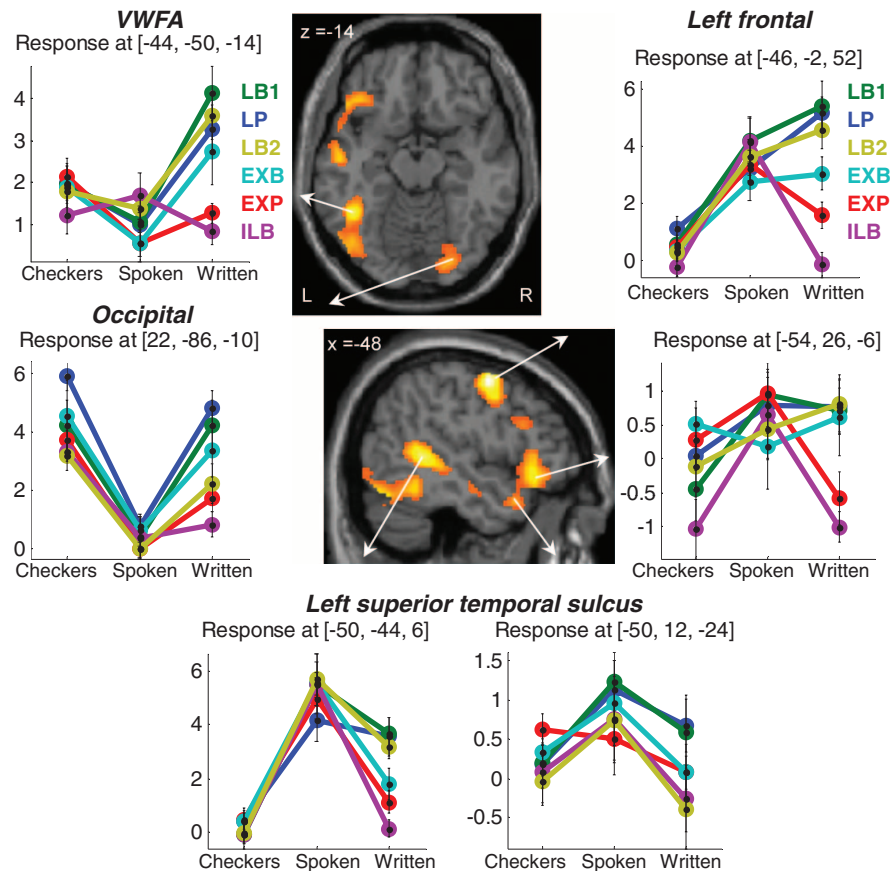
This analysis thus uncovered three simple effects: with the acquisition of literacy, written materials (i) activate right occipital cortex at the same level as checkerboards; (ii) induce a strong activation in left ventral visual cortex, at the classical site of the visual word form area (VWFA); (iii) gain access to left perisylvian temporal and frontal language areas.

**The visual word form area: A major correlate of literacy.** Our next analyses focused on visual responses in the VWFA. The effect of reading performance on occipito-temporal cortex during sentence reading was replicated when passive viewing of letter strings was contrasted to rest [main peak =  $-46, -80, 4$ ,  $Z = 5.75$ ; subpeaks at  $-40, -70, -12$ ,  $Z = 4.50$ ; and the VWFA proper,  $-46, -58, -10$ ,  $Z = 4.11$ ; right occipital region,  $24, -86, -10$ ,  $Z = 5.25$ , corrected  $P < 0.05$  by false detection rate (FDR) analysis (29)]. In this part of the experiment, which involved viewing meaningless pseudowords during an easy target-detection task, only these visual regions were modulated by literacy, confirming their role in automatic orthographic coding (16). Notably, the impact of schooling on the VWFA was replicated when the illiterates were compared with the matched low-SES literates (LB1 < LB2), both for written sentences versus rest ( $-40, -50, -14$ ,  $Z = 6.77$ ) and strings versus rest ( $-48, -60, -10$ ,  $Z = 3.54$ ). The VWFA was also identified when we searched for activation positively correlated with reading performance within the unschooled participants only (illiterates and ex-illiterates; sentences versus rest:  $-42, -54, -6$ ,  $Z = 6.25$ ; strings

versus rest:  $-48, -56, -6$ ,  $Z = 3.53$ ). This finding indicates that adult literacy suffices to establish an increased VWFA response to orthographic patterns.

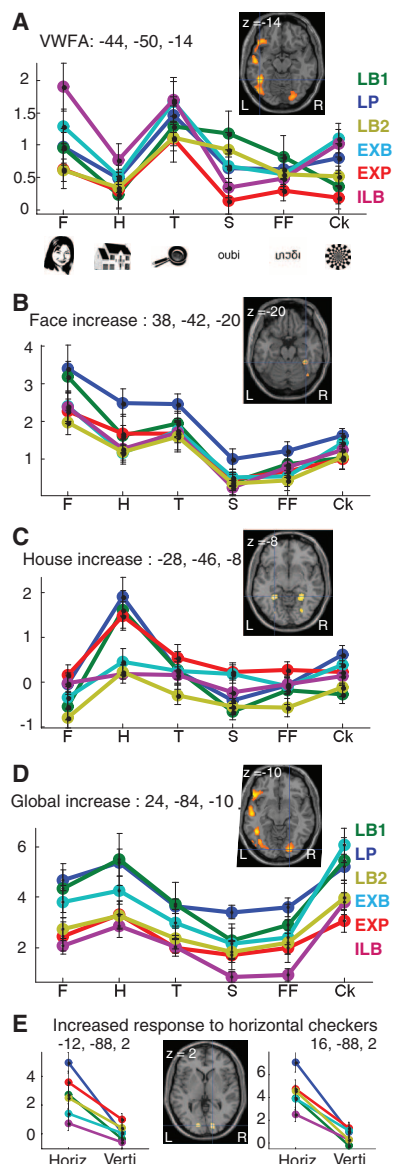
Literacy not only amplified letter string responses, but also increased the cortical selectivity for this category relative to others. When we tested the impact of reading performance on the difference between letter strings and other visual categories, only the VWFA appeared (peak at  $-44, -56, -14$ ,  $Z = 5.00$ ; subpeak  $-44, -68, -12$ ,  $Z = 3.85$ ). This effect showed a highly significant hemispheric asymmetry, peaking at the classical VWFA coordinates ( $-44, -56, -12$ ,  $Z = 5.07$ ). Thus, literacy results in the emergence of a cortical site increasingly more responsive to writing than to other visual categories (17, 30).

Group analyses left open the possibility of a selective but spatially variable response to written strings in every subject. Literacy would then merely displace this response to a reproducible site, without changing its amplitude. This possibility was refuted, however, through an individual analysis in which the voxel most responsive to written sentences versus checkerboards was first identified in each participant, within 10 mm of the group peak (similar results were obtained with 20



**Fig. 2.** Effects of literacy on brain responses to written sentences during the localizer. Axial and sagittal slices show voxels where activation was modulated by literacy during exposure to written sentences relative to rest (voxel  $P < 0.001$ , cluster  $P < 0.05$  corrected). Colored labels refer to participant groups and are ordered according to reading performance (see Fig. 1). Plots report activation to visual checkers, spoken and written language relative to rest in the localizer run, in arbitrary units (mean  $\pm$  1 SE). To avoid circularity, we generated plots solely from the spoken and written commands data, which were independent from the voxel-selection criterion.





**Fig. 3.** Effects of literacy on visual responses to different categories of stimuli. (A) Visual responses in the VWFA. (Inset) Statistical parameter map (SPM) map of modulation by literacy of activation to written sentences in the localizer ( $P < 0.001$ , cluster corrected  $P < 0.05$ ). Plot showing activation in the independent visual runs at the isolated peak (mean  $\pm 1$  SE). Activation increased in response to letter strings ( $P = 0.005$ ) and decreased for checkerboards ( $P = 0.013$ ) and, to a lesser extent, for faces (overall  $P = 0.09$ ,  $ILL > LB2$ ,  $P = 0.003$ ). (B and C) SPM maps (voxel  $P < 0.001$  uncorrected) and peak plots showing increases with reading performance of face responses in right anterior fusiform, and of house responses in bilateral parahippocampal regions. (D) SPM map of enhancement by literacy of the response to written sentences in lateral occipital cortex (inset,  $P < 0.001$ , cluster corrected  $P < 0.05$ ), and plot showing its replication in response to other visual categories. (E) Modulation by literacy of the greater response to horizontal than vertical checkerboards in primary visual cortex (inset, voxel  $P < 0.001$  uncorrected). In (B), (C), and (E), plots are provided for illustration, because they do not arise from independent data; plots (A) and (D) are from independent data.

or 40 mm), and then analyzed for its responses to strings and false fonts in the independent visual runs. We observed a significant lateral-to-mesial shift of the word-responsive peak (from  $x = -48$  in illiterates to  $-46$  in ex-illiterates and  $-44$  in literates,  $P = 0.006$ ), but its activation to strings also increased strongly with reading performance (linear regression,  $r^2 = 46.5\%$ ,  $P < 0.0001$ ), as did the selectivity index for strings relative to false fonts ( $P = 0.001$ ). Thus, literacy genuinely increases both the strength and specificity of cortical responses to the learned script in the VWFA.

We also searched for nonmonotonic effects across our six groups, which might arise if the ex-illiterates had to mobilize a broader network than either literates or illiterates in order to read. Indeed, during sentence reading, Brazilian ex-illiterates showed greater activity than Brazilian literates in bilateral mesial fusiform areas ( $-34, -60, 0$ ,  $Z = 5.34$ ;  $38, -50, -2$ ,  $Z = 4.54$ ) and right posterior parietal cortex ( $24, -62, 38$ ,  $Z = 4.70$ ;  $12, -58, 60$ ,  $Z = 4.16$ ) (fig. S5). Thus, to achieve their modest reading performance, ex-illiterates engage a broader and more bilateral ventral network than literates and recruit additional posterior parietal regions associated with serial effortful reading (31). This observation is similar to the developmental finding that reading in young children initially involves a broad bilateral visual network (18) that progressively restricts to the VWFA as greater expertise sets in (19).

**Competition with other visual categories in occipito-temporal cortex.** At the peak coordinates of the VWFA ( $-44, -50, -14$ , identified by the localizer), analysis of the independent visual runs showed a strong response to strings but also to other visual categories, particularly faces and tools (Fig. 3). This finding confirms that this area plays a broad role in visual shape analysis. Reading being a recent invention, we expected that written words would not activate a fully dedicated cortical site but would only partially “re-cycle” existing cortical mechanisms for visual recognition (2, 23), inducing a cortical competition that would increase with reading expertise. We tested the predicted cortical competition by searching for a decreasing response to visual stimuli with increasing reading performance. At the independently defined VWFA peak, the responses to checkerboards slightly diminished with reading performance, both across all groups (linear regression,  $P = 0.013$ ), for illiterates compared to all other groups ( $P = 0.025$ ), and for illiterates compared to SES-matched literates ( $ILB > LB2$  comparison,  $P = 0.039$ ). For houses and tools, only marginal decreasing trends were found (same three tests,  $P = 0.08, 0.025, 0.09$  for houses and  $0.13, 0.025, 0.06$  for tools). For faces, the decreasing tendency was stronger: The regression with reading performance across all participants was marginal ( $P = 0.09$ ), but the more focused comparisons were significant ( $ILB > \text{other groups}$ ,  $P = 0.025$ ;  $ILB > LB2$ ,  $P = 0.003$ ). Comparing illiterates with SES-matched literates indeed arguably provides a purer test, controlling for the pos-

sibility that frequency of exposure to faces might increase with socioeconomic status and influence fusiform responses (32). When studied at the whole-brain level, the  $ILB > LB2$  contrast indicated a highly significant reduction of face response with literacy ( $P < 0.001$ , cluster  $P < 0.05$  corrected) in two bilateral posterior fusiform clusters (right:  $40, -80, 0$ ,  $Z = 5.93$ , with an anterior subpeak,  $38, -50, -12$ ,  $Z = 4.70$ ; left,  $-44, -70, -12$ ,  $Z = 4.58$ , with a subpeak precisely at the VWFA,  $-42, -54, -14$ ,  $Z = 3.91$ ). The same contrast did not reach corrected-level significance for houses or tools.

In summary, at the VWFA site, learning to read competes primarily with the cortical representation of checkers and faces. Further analyses showed that this competition was spatially restricted. We implemented analyses inspired by Golarai *et al.* (28), who showed that cortical peaks with adult-like selectivity to faces and places already exist in 7 to 11 year olds and, with increasing age, progressively expand into the surrounding cortex. For each subject, we first searched for the peak response to faces versus houses within 10 mm of the group coordinates of the VWFA. We then examined an orthogonal regression testing how the activation to faces varied with literacy, at the peak and in increasingly larger annuli of 2, 4, 6, or 8 voxels surrounding it (fig. S6). There was no change in peak face responses with reading performance ( $P = 0.47$ ), nor in annuli of radius 2 or 4, but in the more distant annuli of radius 6 or 8, face activation decreased with reading performance (regression across all groups, respectively  $P = 0.037$  and  $P = 0.015$ ). Similar findings were obtained for the individual peak of responsivity to houses versus faces: no change in peak activation ( $P = 0.20$ ), but a decrease in house-driven activation in the larger annulus of radius 8 ( $P = 0.045$ ). For tools, a category for which no selective region exists in ventral visual cortex (33), we did not find an annular reduction, but a more diffuse reduction in activation with reading performance, significant over both a large sphere of 16 mm ( $P = 0.039$ ) and in the 50 voxels best responsive to tools versus houses ( $P = 0.019$ ), but again not at the peak itself ( $P = 0.27$ ).

Overall, our results indicate that the developmental competition induced by the expansion of orthographic representations in the ventral visual system is modest, does not directly affect the peak responses to faces and houses, but interferes with their expansion into the surrounding cortex. These conclusions fit with previous studies of visual development (28), expertise (24, 25), and plasticity of sensory maps (20), which reveal a displacement of map boundaries due to cortical competition.

**Positive effects of literacy on visual organization.** Competition could also have a positive effect on cortical responses to non-reading-related visual categories: By reducing the dispersion of their neural responses, it might force them to a more consistent cortical site. Indeed, a whole-brain search revealed positive correlations of reading performance with face and house responses in ventral occipito-temporal cortex (Fig. 3). Reading

performance modulated positively the face-versus-rest contrast in the right anterior fusiform gyrus (38, -42, -20,  $Z = 4.84$ , FDR) and induced a significant right-hemispheric shift of face responses in occipito-temporal cortex (24, -88, -10,  $Z = 8.02$ ; 36, -62, -12,  $Z = 6.72$ ; and 38, -40, -22,  $Z = 4.77$ , FDR). Similarly, reading performance modulated the house-versus-rest contrast in bilateral mesial fusiform and parahippocampal regions (peaks at 34, -58, -12,  $Z = 5.19$ ; 24, -36, -16,  $Z = 4.83$ ), with a right-hemispheric asymmetry (36, -60, -12,  $Z = 5.53$ ). Altogether, these influences on word, face, and place responses resulted in a better differentiated mosaic of category-specific regions in ventral visual cortex in literates (Fig. 4). Notably, however, face and house increases were found neither when we compared illiterates with their SES-matched literate group (ILB < LB2 comparison), nor when we tested for the effect of literacy in nonschooled participants only (table S2). Plots showed that these effects differentiated the participants living primarily in urban areas (LB1, LP, and to a lesser extent, EXP) versus those living in rural areas (ILB, EXB, and LB2), regardless of their schooling and reading scores (Fig. 3). Although these observations suggest an influence of familiarity rather than literacy or schooling per se, they are nevertheless important in showing how ventral fusiform organization can be affected by cultural variables (1).

Literacy led to another effect: a general enhancement of occipital responses. We probed the right occipital location identified as being modulated by reading performance during written sentences versus rest in the localizer run (coordinates 22, -86, -10). During the independent visual runs, the activation of this region to every visual category correlated positively with reading scores, with the lowest correlation achieved with the checkerboards ( $r^2 = 0.08$ ,  $P = 0.02$ ; all other categories,  $r^2$  ranging from 0.17 to 0.22,  $P < 0.0007$ ). Furthermore, these effects were genuinely related to literacy, not just schooling (table S2). When extended to a whole-brain search, with a main contrast for increasing activation to all visual categories, this effect was significant not only in right occipital (24, -84, -10,  $Z = 14.6$ ) but also in left occipital cortex (-48, -80, -4,  $Z = 9.35$ ) and a right occipito-parietal cluster (24, -76, 36,  $Z = 6.75$ ), always with significant right-hemispheric asymmetry. Thus, literacy enhanced occipital responses to essentially all the contrasted black-and-white visual stimuli used in our study.

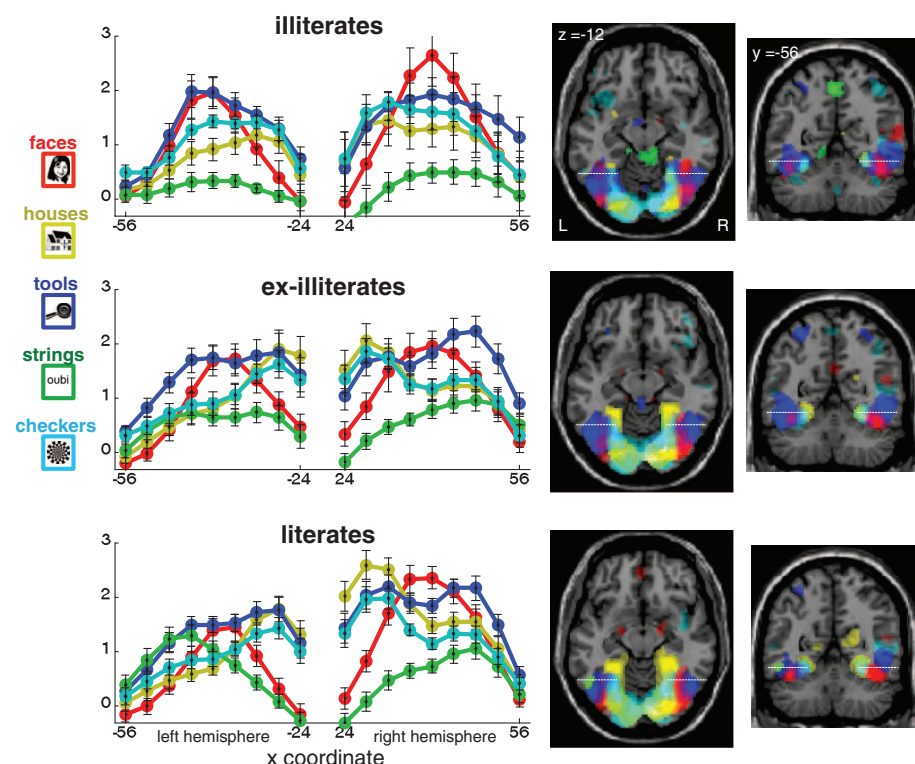
We also examined whether early retinotopic responses were affected. The localizer comprised horizontal and vertical checkerboards, designed to isolate the meridians of early visual maps. In the Roman alphabet, words appear as horizontally extended strings, and expert readers show enhanced behavioral processing of letter strings presented at the familiar foveal and horizontal location (31, 34). We therefore predicted that literacy might increase the responses to horizontal relative to vertical checkerboards. Indeed, this effect was observed at two symmetrical occipital

sites corresponding to primary visual cortex (16, -88, 2,  $Z = 5.10$ ; and -12, -88, 2,  $Z = 4.75$ , FDR). These sites exhibited a strong response to horizontal, but not vertical checkerboards, and the modulation by reading performance was seen only with horizontal checkerboards (Fig. 3). This effect was significantly stronger in left than in right V1 (asymmetry effect for horizontal > vertical checkerboards, peak at -4, -88, -4,  $Z = 5.37$ , FDR). Both occipital sites also showed a positive correlation with literacy when written sentences were presented (table S2). Overall, these results suggest that literacy results in a form of perceptual learning (20–22) that refines the earliest stage of cortical visual processing. At this stage, learning is generic enough to generalize to checkerboard stimuli presented at the trained location. The greater effect in left area V1 fits with the larger letter-identification span in the right visual field in left-to-right readers (35).

**Enhanced responses to spoken language.** Finally, we examined how literacy affected spoken language processing (Fig. 5). Several regions showed a decreasing activation to spoken sentences with greater reading performance: left posterior STS (-44, -52, 18,  $Z = 5.45$ ), left and right middle temporal gyri (-66, -22, -10,  $Z = 5.25$ ; 48, -30, -8,  $Z = 5.34$ ), and midline anterior cingulate cortex (4, 42, 42,  $Z = 4.11$ ). These reductions

probably reflect a facilitation of speech comprehension in literate participants (8). In the converse direction, however, activation to spoken sentences essentially doubled from illiterates to literates in left and right superior temporal regions just posterior to Heschl's gyrus (planum temporale; -38, -28, 18,  $Z = 5.52$ ; 42, -14, 16,  $Z = 5.43$ ), with bilateral subpeaks near Heschl's gyrus (-60, -14, 10;  $Z = 4.28$ ; 66, -2, 24,  $Z = 4.41$ ) and a significant left-hemispheric asymmetry. The effect was replicated in the independent auditory lexical decision runs, with both words and pseudo-words (correlations with reading performance:  $r^2 = 0.20$ ,  $P = 0.0002$ ; and  $r^2 = 0.18$ ,  $P = 0.0005$ ). The enhanced temporal response was restricted to spoken language, with no trace of activation to written sentences at this site.

The planum temporale is involved in phonological coding of speech (36) and is sensitive to the congruity between a speech sound and a simultaneous visually presented letter (37), an effect that is reduced or absent in dyslexic subjects (38). Our results make this region a prime candidate for the enhanced phonemic processing that accompanies reading acquisition [(10–12); see also (39)]. They also suggest that the reduced planum temporale activation seen in dyslexic children, rather than being a cause of dyslexia (38), could be a consequence of abnormal reading acquisition.



**Fig. 4.** Mosaic of preferences for different visual categories in ventral visual cortex. Slices at right show the activation difference between a given category and all the others [for greater comparability between groups with different numbers of subjects, the figure does not show statistical  $t$  maps, but blood oxygen level-dependent (BOLD) signal maps arbitrarily thresholded at 0.66% of the mean BOLD signal over the whole brain; similar results were seen with  $t$  maps]. Graphs at left show the evolution of the signal relative to rest for the different categories (mean  $\pm$  1 SE, at successive cortical sites tracing a horizontal line through the classical coordinates of the VWFA (-42, -57, -12; dotted line).



During auditory lexical decision, but not sentence listening, a second site in left inferior temporal cortex also increased its activation as a function of reading performance ( $-48, -52, -8$ ,  $Z = 8.62$ ). This site showed no activation in illiterates, but a strong one in all literate groups (Fig. 5). Its coordinates strongly suggest a top-down activation of the VWFA and the neighboring lateral inferior temporal cortex (40). Indeed, its activation to spoken words and pseudowords was positively correlated to its activation by written strings in the independent visual runs ( $r = +0.46$ ; no such correlation was found with other visual categories). Overlap with the VWFA was also established by first identifying the peak response to written sentences versus checkerboards in each participant, and then correlating its activation during spoken lexical decision with the participant's reading performance ( $P = 0.005$ ). Altogether, those results confirm that the VWFA can be activated in a top-down manner during speech processing (41–43), even in a lexical decision task that does not require orthographic processing. Because this activation is present only inasmuch as the participants can read, our findings suggest that it reflects the recruitment of

an orthographic code rather than a generic picture code (44).

Previous psycholinguistic research has demonstrated that orthography affects spoken language processing (11–14), but it remains debated whether an orthographic code is activated online whenever we hear a spoken word, or whether orthography merely changes the nature of phonological representations (12). Our results show that both phenomena coexist: The planum temporale increase suggests enhanced phonological coding, compatible with a recent study using low-resolution electroencephalography (12), whereas the VWFA activation indicates an additional and optional orthographic recruitment.

#### Effects of early schooling and late literacy.

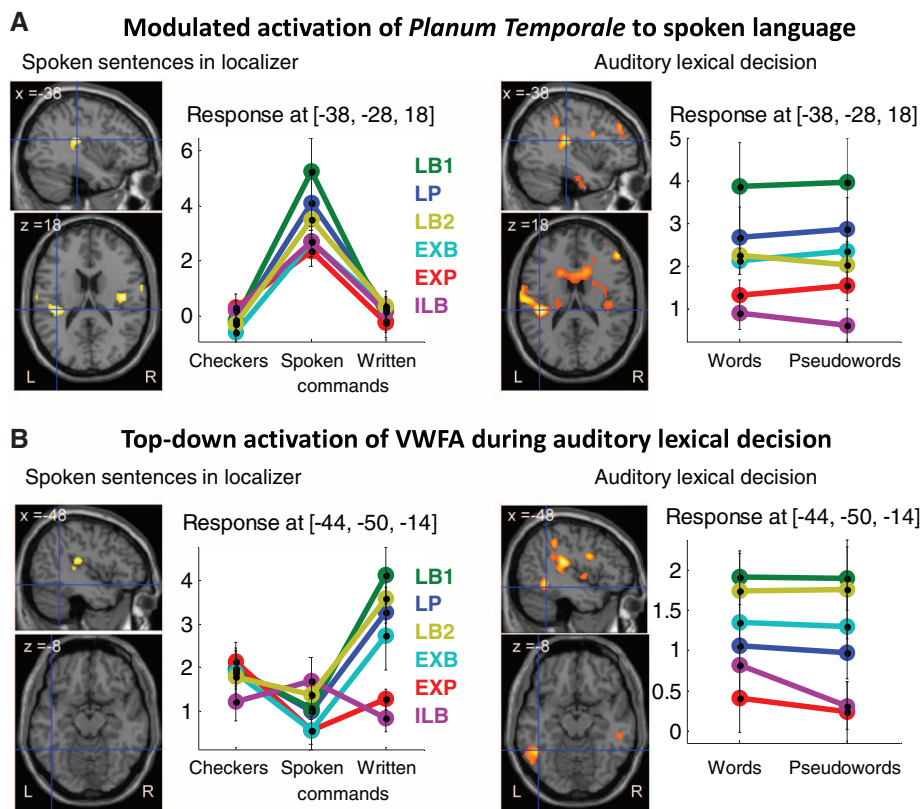
The above fMRI findings are based on global correlations of brain activation with reading performance, and therefore reflect the joint influences of schooling and literacy. To separate these variables, we performed additional regression analyses on all previously identified peaks (29). One analysis evaluated the impact of literacy acquired during adulthood by testing the effect of reading performance within unschooled subjects only. Another analysis, conversely, probed the impact

of early schooling by regressing out the effect of reading performance and testing for a remaining difference between the Brazilian ex-illiterates (EXB) and the low-SES Brazilian literates (LB2 group), who only differed in early schooling.

The results were clear-cut. Essentially all of the above effects of literacy were present in ex-illiterates who learned to read during adulthood (table S2). Such was the case for the increased VWFA response to letter strings; the capacity to activate the spoken language network through reading (except in left temporal pole); the general visual increase in right occipital cortex; the greater response of area V1 to horizontal checkerboards and written sentences; and the enhanced planum temporale and top-down VWFA activation to spoken words and pseudowords. Thus, the neural modifications induced by adult literacy education were considerable. Furthermore, the vast majority were unaffected by early schooling (table S2). There were only two interesting exceptions. The first was the reduced activation to faces in the VWFA, which was indeed particularly prominent in the LB2 group, who benefited from early schooling, relative to the EXB group. This finding suggests that competitive interactions between written words and faces in ventral visual cortex primarily occur when reading is acquired in childhood, a time when visual maps are known to be highly malleable (28). The other effect of early schooling concerned a marginal left pre-motor increase in activation to written sentences. This region overlaps with Exner's writing center and is thought to code handwriting gestures (45). Whereas early-schooled participants were fluent in handwriting, it is possible that the ex-illiterates did not receive enough training to automatically activate a gesture code from the mere vision of written sentences.

We caution that these conclusions may only be valid for the moderate level of reading fluency achieved by our ex-illiterate participants. Whether early-schooling effects truly reflect a limit on adult sensory plasticity or would vanish with more intense reading practice remains an open question. However, our results also indicate that, in literates, most of the observed effects do not change further as reading expertise increases (table S2; the only exceptions were increases in planum temporale and left pSTS, and reduced VWFA activation to checkerboards). In particular, the VWFA activation to words and strings increases briskly from illiterates to ex-illiterates and then reaches a plateau uncorrelated with ultimate proficiency, in agreement with developmental evidence that minimal literacy training suffices to establish it in 6 year olds (18).

**Conclusion.** Literacy, whether acquired in childhood or through adult classes, enhances brain responses in at least three distinct ways. First, it boosts the organization of visual cortices, particularly by inducing an enhanced response to the known script at the VWFA site in left occipito-temporal cortex and by augmenting early visual responses in occipital cortex, in a partially retino-



**Fig. 5.** Effects of literacy on brain responses to spoken language. (A) SPM maps of the effect of literacy on the activation to spoken sentences (left), words and pseudowords (right; voxel  $P < 0.001$ , cluster-size corrected  $P < 0.05$ ). Plots show the effect observed in the left planum temporale: Activation to spoken language, words, and pseudowords was doubled or more after the acquisition of literacy (peak selected using the localizer run, left panel, and replicated in the independent lexical decision runs, right panel). (B) SPM maps of the effect of literacy on activations in ventral occipito-temporal cortex to spoken language. Literacy modulated activation to words and pseudowords during auditory lexical decision (right), but not during mere listening to spoken commands. Plots show the response at the a priori location of the VWFA, as defined by the effect of literacy on written sentences (same location as in Figs. 2 and 3A).

topic manner. Second, literacy allows practically the entire left-hemispheric spoken language network to be activated by written sentences. Thus reading, a late cultural invention, approaches the efficiency of the human species' most evolved communication channel, namely speech. Third, literacy refines spoken language processing by enhancing a phonological region, the planum temporale, and by making an orthographic code available in a top-down manner. These largely positive changes should not hide that literacy, like other forms of expertise, also leads to cortical competition effects (23–26). At the VWFA site, a significantly reduced activation was found for checkerboards and faces. The intriguing possibility that our face perception abilities suffer in proportion to our reading skills will be explored in future research.

## References and Notes

- J. Henrich, S. J. Heine, A. Norenzayan, *Behav. Brain Sci.* **33**, 61 (2010).
- S. Dehaene, L. Cohen, *Neuron* **56**, 384 (2007).
- A. Castro-Caldas et al., *Eur. J. Neurol.* **6**, 23 (1999).
- M. Carreiras et al., *Nature* **461**, 983 (2009).
- K. M. Petersson, C. Silva, A. Castro-Caldas, M. Ingvar, *Eur. J. Neurosci.* **26**, 791 (2007).
- G. Li et al., *Hum. Brain Mapp.* **27**, 144 (2006).
- K. M. Petersson, A. Reis, S. Askellöf, A. Castro-Caldas, M. Ingvar, *J. Cogn. Neurosci.* **12**, 364 (2000).
- A. Castro-Caldas, K. M. Petersson, A. Reis, S. Stone-Elander, M. Ingvar, *Brain* **121**, 1053 (1998).
- K. M. Petersson, A. Reis, A. Castro-Caldas, M. Ingvar, *Neuroimage* **10**, 45 (1999).
- J. Morais, P. Bertelson, L. Cary, J. Alegria, *Cognition* **24**, 45 (1986).
- H. Cheung, H. C. Chen, *Lang. Cogn. Process.* **19**, 1 (2004).
- L. Perre, C. Pattamadilok, M. Montant, J. C. Ziegler, *Brain Res.* **1275**, 73 (2009).
- P. Ventura, J. Morais, R. Kolinsky, *Cognition* **105**, 547 (2007).
- J. C. Ziegler, A. Petrova, L. Ferrand, *J. Exp. Psychol. Learn. Mem. Cogn.* **34**, 643 (2008).
- B. A. Shaywitz et al., *Biol. Psychiatry* **52**, 101 (2002).
- L. Cohen, S. Dehaene, *Neuroimage* **22**, 466 (2004).
- C. I. Baker et al., *Proc. Natl. Acad. Sci. U.S.A.* **104**, 9087 (2007).
- S. Brem et al., *Proc. Natl. Acad. Sci. U.S.A.* **107**, 7939 (2010).
- U. Maurer et al., *Neuroimage* **33**, 749 (2006).
- D. B. Polley, E. E. Steinberg, M. M. Merzenich, *J. Neurosci.* **26**, 4970 (2006).
- W. Li, V. Piëch, C. D. Gilbert, *Nat. Neurosci.* **7**, 651 (2004).
- M. Sigman et al., *Neuron* **46**, 823 (2005).
- S. Dehaene, *Reading in the Brain* (Penguin Viking, New York, 2009).
- I. Gauthier, T. Curran, K. M. Curby, D. Collins, *Nat. Neurosci.* **6**, 428 (2003).
- A. Harel, S. Gilaie-Dotan, R. Malach, S. Bentin, *Cereb. Cortex* **20**, 2304 (2010).
- B. Rossion, C. C. Kung, M. J. Tarr, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 14521 (2004).
- D. J. Bolger, C. A. Perfetti, W. Schneider, *Hum. Brain Mapp.* **25**, 92 (2005).
- G. Golarai et al., *Nat. Neurosci.* **10**, 512 (2007).
- Materials and methods are available as supporting material on Science Online.
- R. Gaillard et al., *Neuron* **50**, 191 (2006).
- L. Cohen, S. Dehaene, F. Vinckier, A. Jobert, A. Montavont, *Neuroimage* **40**, 353 (2008).
- E. Eger, S. R. Schweinberger, R. J. Dolan, R. N. Henson, *Neuroimage* **26**, 1128 (2005).
- P. E. Downing, A. W. Chan, M. V. Peelen, C. M. Dodds, N. Kanwisher, *Cereb. Cortex* **16**, 1453 (2006).
- T. A. Nazir, N. Ben-Boutayab, N. Decoppet, A. Deutsch, R. Frost, *Brain Lang.* **88**, 294 (2004).
- K. Rayner, *Psychol. Bull.* **124**, 372 (1998).
- C. Jacquemot, C. Pallier, D. LeBihan, S. Dehaene, E. Dupoux, *J. Neurosci.* **23**, 9541 (2003).
- N. van Atteveldt, E. Formisano, R. Goebel, L. Blomert, *Neuron* **43**, 271 (2004).
- V. Blau et al., *Brain* **133**, 868 (2010).
- P. E. Turkeltaub, L. Gareau, D. L. Flowers, T. A. Zeffiro, G. F. Eden, *Nat. Neurosci.* **6**, 767 (2003).
- L. Cohen, A. Jobert, D. Le Bihan, S. Dehaene, *Neuroimage* **23**, 1256 (2004).
- Y. N. Yoncheva, J. D. Zevin, U. Maurer, B. D. McCandliss, *Cereb. Cortex* **20**, 622 (2010).
- A. S. Desroches et al., *Brain Res.* **1356**, 73 (2010).
- J. R. Booth et al., *Hum. Brain Mapp.* **19**, 155 (2003).
- F. Kherif, G. Josse, C. J. Price, *Cereb. Cortex* **10.1093/cercor/bhq063** (2010).
- M. Longcamp, J. L. Anton, M. Roth, J. L. Velay, *Neuroimage* **19**, 1492 (2003).
- This work was supported by Institut National de la Santé et de la Recherche Médicale (INSERM), Commissariat à l'Energie Atomique (CEA), Collège de France, Agence Nationale de la Recherche (project CORELEX), Fundação para a Ciência e a Tecnologia, the European Community FEDER funding (Project PTDC/PSI/66077/2006, "Cognitive consequences of literacy"), SARAH Network of Rehabilitation Hospitals, and by two grants from the Belgian French community (Fonds de la Recherche Fondamentale Collective 2.4586.07 and Action de Recherche Concertée 06/11-342). We gratefully acknowledge A. Amadon, M.-H. Baju, L. Labruna, D. Le Bihan, L. Hertz-Pannier, P. Pinel, and the NeuroSpin infrastructure groups at NeuroSpin; C. Carvalho, L. Querido, S. Fernandes, and T. Fernandes at the Faculty of Psychology, University of Lisbon; and D. Y. Suguieda, R. S. Vera, A. G. Tauil, E. Amemiya, L. G. N. Nunes, S. B. de Oliveira, and C. L. M. Gillis at SARAH Brasília.

## Supporting Online Material

[www.sciencemag.org/cgi/content/full/science.1194140/DC1](http://www.sciencemag.org/cgi/content/full/science.1194140/DC1)  
Materials and Methods  
SOM Text  
Figs. S1 to S8  
Tables S1 and S2  
References

23 June 2010; accepted 20 October 2010  
Published online 11 November 2010;  
10.1126/science.1194140

# REPORTS

## Carbon Nanotubes with Temperature-Invariant Viscoelasticity from $-196^{\circ}$ to $1000^{\circ}\text{C}$

Ming Xu,<sup>1</sup> Don N. Futaba,<sup>1\*</sup> Takeo Yamada,<sup>1</sup> Motoo Yumura,<sup>1</sup> Kenji Hata<sup>1,2\*</sup>

Viscoelasticity describes the ability of a material to possess both elasticity and viscosity. Viscoelastic materials, such as rubbers, possess a limited operational temperature range (for example, for silicone rubber it is  $-55^{\circ}$  to  $300^{\circ}\text{C}$ ), above which the material breaks down and below which the material undergoes a glass transition and hardens. We created a viscoelastic material composed from a random network of long interconnected carbon nanotubes that exhibited an operational temperature range from  $-196^{\circ}$  to  $1000^{\circ}\text{C}$ . The storage and loss moduli, frequency stability, reversible deformation level, and fatigue resistance were invariant from  $-140^{\circ}$  to  $600^{\circ}\text{C}$ . We interpret that the thermal stability stems from energy dissipation through the zipping and unzipping of carbon nanotubes at contacts.

Viscoelasticity describes the ability of a material to both dissipate energy (viscous) and reversibly deform (elastic) and permeates all levels of our lives from human tissues, shoe soles, ear plugs, and mattresses to vibration isolators. Viscoelastic properties of existing ma-

terials, represented by rubbers, are inherently temperature dependent (*1*). This is because molecular motion that is the origin of viscoelasticity is a thermally activated process.

Carbon nanotubes (CNTs), with their exceptional mechanical properties and thermal stability

(2), could be building blocks for various thermally stable elastic and viscoelastic materials. Aligned, sparse CNT arrays (3), films (4), and sponges packed with short, straight CNTs (5) have shown fatigue resistance, supercompressibility, and compressive elasticity, respectively. Reports of creep and buckling of aligned CNTs (6, 7) demonstrate that a specific assembly of CNTs can show viscoelasticity.

Our strategy to make a viscoelastic CNT material was to assemble traversing long CNTs with a very high density of intermittent physical interconnections, analogous to an aggregate of hair. We made a CNT network where each CNT made contacts with numerous other CNTs. A combination of reactive ion etching (RIE) to the catalyst film, water-assisted chemical vapor deposition (8), and compression was used for the

<sup>1</sup>Technology Research Association for Single Wall Carbon Nanotubes (TASO) and Nanotube Research Center, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, 305-8565, Japan. <sup>2</sup>Japan Science and Technology Agency (JST), Kawaguchi, 332-0012, Japan.

\*To whom correspondence should be addressed. E-mail: kenji-hata@aist.go.jp (K.H.); d-futaba@aist.go.jp (D.N.F.)



synthesis. RIE exposure reduced the catalyst density needed to create randomly oriented and sparse CNTs. Water-assisted chemical vapor deposition synthesized very long and clean CNTs ( $\sim 68\%$  double-walled CNTs,  $\sim 22\%$  single-walled CNTs, and  $\sim 10\%$  tripled-walled CNTs;  $0.009 \text{ g/cm}^3$ ; height =  $4.5 \text{ mm}$ ; carbon purity =  $99.9\%$ ) that are essential to increase interconnection among CNTs and thereby increase cohesiveness and thermal stability. Compression increased the CNT material density fourfold ( $0.036 \text{ g/cm}^3$ ) (Fig. 1A).

Scanning electron microscope (SEM) observation of the CNT material revealed an intertube structure where individual CNTs traversed laterally, making interconnections with other CNTs. This feature allowed the CNT material to bear strain without fracture (Fig. 1B). The slow elastic recovery after release implied viscoelasticity. Stress-strain behavior from shear-mode dynamic mechanical analysis (DMA) showed up to  $100\%$  strain, high nonlinearity, and a closed hysteresis without abrupt changes, which were typical of viscoelastic, energy-dissipative, and highly deformable materials, for example, rubber. Silicone rubber was chosen as our strictest benchmark in terms of thermal stability because it is the most thermally resistant rubber. The observed dual slope of the stress-strain curve was associated

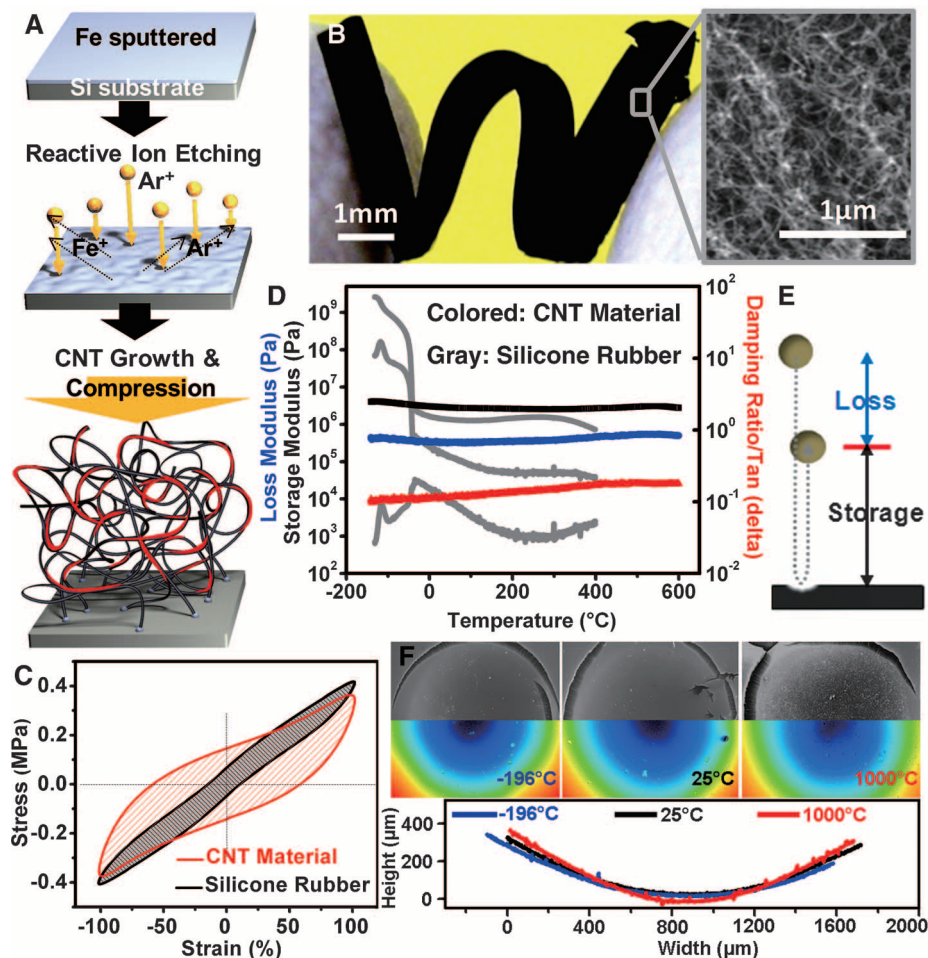
with a change of the modulus as we approached the failure strain. The larger enclosed area of the hysteresis loop for the CNT material meant more energy dissipated in one cycle (Fig. 1C). Quantitatively, the viscoelastic properties (storage modulus, loss modulus, and damping ratio) by DMA showed that the CNT material possessed similar stiffness (storage modulus =  $1 \text{ MPa}$ ) and higher dissipation ability (loss modulus =  $0.3 \text{ MPa}$ ) and damping ratio ( $0.3$ ) than silicone rubber at room temperature (fig. S6A). The viscoelastic properties of the CNT material strongly depend on the internal structure. For example, an increase in density results in a significant increase in both moduli. We compressed the as-prepared CNT material to increase the density four times, and this resulted in an increase of the storage ( $5$  times) and loss ( $10$  times) moduli and the damping ratio ( $2$  times). In this way, our CNT material was specifically tuned to exhibit similar viscoelastic properties as silicone rubber at room temperature.

The viscoelastic properties (storage modulus, loss modulus, and damping ratio) measured by DMA in ambient  $\text{N}_2$  were nearly constant over an exceptionally wide temperature range ( $-140^\circ$  to  $600^\circ\text{C}$ ) in contrast to silicone rubber, which showed large variation because of hardening at  $-55^\circ\text{C}$  and degradation at  $300^\circ\text{C}$  (Fig. 1D). Variation between CNT samples was within  $\sim 5\%$ .

As far as we know, commercial DMAs are limited to  $600^\circ\text{C}$ . As exemplified by the vibration isolator demonstration (fig. S2), the CNT material showed viscoelasticity beyond the limitation at  $-190^\circ\text{C}$  (immersed in liquid nitrogen) and at  $>900^\circ\text{C}$  (exposed to butane torch). To extend the temperature range studied, we implemented impact tests at  $-196^\circ\text{C}$ ,  $25^\circ\text{C}$ , and  $1000^\circ\text{C}$  by using a steel ball and analyzed the ball tracks. The ball tracks were identical for all cases as observed by SEM and three-dimensional (3D) mapping (Fig. 1, E and F), which suggested unvarying viscoelastic properties across this  $1200^\circ\text{C}$  temperature range. The absence of catalyst materials in our CNT material is crucial for our high thermal stability because oxidation would always limit the practical applications in air above  $\sim 400^\circ\text{C}$ , particularly when catalyst material is present (9).

Further DMA characterization from  $-140^\circ$  to  $600^\circ\text{C}$  demonstrated temperature-invariant frequency stability, the same level of reversible deformation, and fatigue resistance. Frequency-dependence test (Fig. 2, A to C) showed constant viscoelastic properties (storage modulus, loss modulus, and damping ratio) of the CNT material in the range of  $0.1$  to  $100 \text{ Hz}$ . Furthermore, the CNT material showed identical frequency-stability from  $-140^\circ$  to  $600^\circ\text{C}$ .

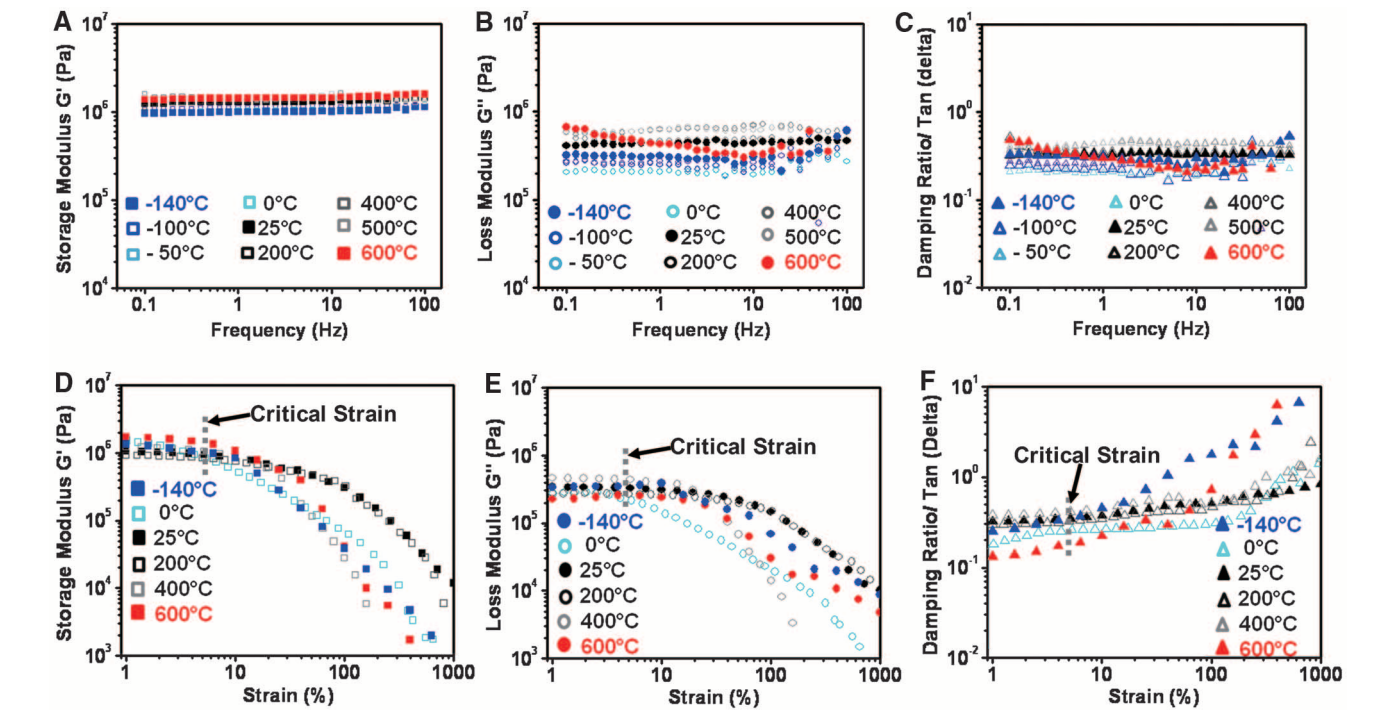
**Fig. 1.** Invariant energy dissipation of the CNT material, from  $-196^\circ$  to  $\sim 1000^\circ\text{C}$ . (A) Diagram showing the synthesis procedure. (B) Photograph of the flexible CNT material. (Inset) SEM image. (C) Stress-strain curves of CNT material and silicone rubber. (D) Temperature dependence of the storage modulus (black), loss modulus (blue), and damping ratio (red) of the CNT material (silicone rubber in gray for comparison). (E) Schematic of the impact test. (F) Split images of the ball tracks performed at  $-196^\circ$ ,  $25^\circ$ , and  $1000^\circ\text{C}$  (top, SEM; bottom, 3D mapping from laser microscopy). Bottom graph, all track profiles for all three cases.



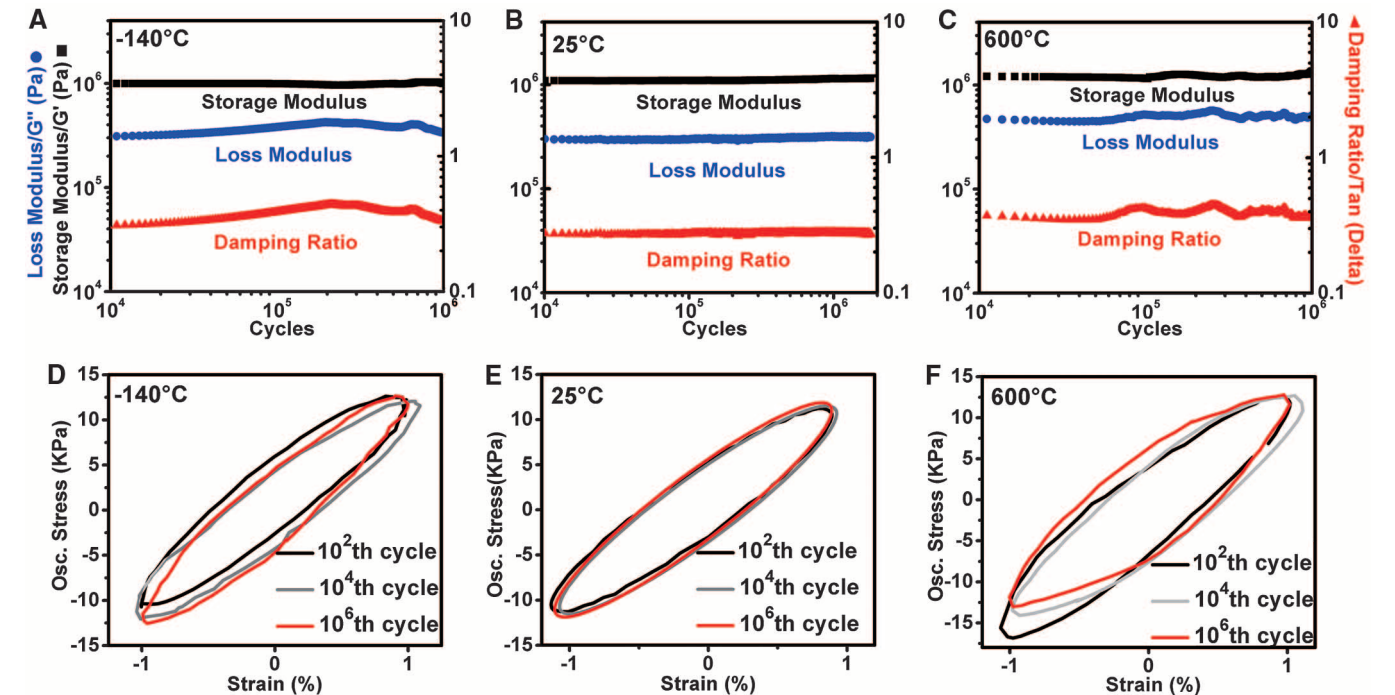
Strain dependence tests (Fig. 2, D to F) showed that the critical strain, that is, the maximum strain allowing reversible deformation, was ~5%. Similarly, the CNT material retained the same level of reversible deformation from -140° to 600°C. The failure strain for the CNT material

at room temperature was estimated as ~100% (fig. S6B). At the failure strain, the structure broke down, indicated by the degradation in the non-linearity and hysteresis loops at 100% strain (fig. S9). The failure strain ranged from 50 to 100% between -140°C and 600°C, which we suspect

results from the instability of gap between pressure heads at large strain because of thermal expansion and contraction. Cyclic tests at 100 Hz showed identical viscoelastic moduli and stress-strain behavior even after 1,000,000 cycles at 1% strain (Fig. 3, B and



**Fig. 2.** Viscoelastic properties of the CNT material over a wide temperature range. (A to C) Storage modulus, loss modulus, and damping ratio of the CNT materials as function of frequency (0.1 to ~100 Hz) at temperatures from -140° to 600°C. (D to F) Storage modulus, loss modulus, and damping ratio of the CNT materials as function of strain amplitude (1 to ~1000%) at temperatures from -140° to 600°C.



**Fig. 3.** Fatigue resistance of the CNT material across -140° to 600°C. Cyclic test (1% strain, 100 Hz, 10<sup>6</sup> cycles) at (A) -140°C, (B) 25°C, and (C) 600°C. Stress-strain curve of fatigue resistance test (10<sup>2</sup>-th 10<sup>4</sup>-th, 10<sup>6</sup>-th cycle) at (D) -140°C, (E) 25°C, and (F) 600°C.



E) that proved excellent fatigue resistance of the CNT material at room temperature. Identical fatigue resistance was observed at  $-140^{\circ}\text{C}$  and  $600^{\circ}\text{C}$ , as evidenced by similar viscoelastic moduli and cyclic behavior (Fig. 3, A, C, D, and F). This phenomenon means that not only are the viscoelastic properties temperature invariant but also unvarying over many cycles.

The porous nature of the CNT material allows for rapid and efficient heat dissipation, which prevents significant heat accumulation, the common cause for property degradation. We interpret that our CNT material exhibited this high level of reversible deformation from the entanglement of the long traversing CNTs throughout the material, like a network of springs creating elasticity.

To provide insight into the mechanism of viscoelasticity, we carried out SEM observations (Fig. 4A) and Herman's orientation factor (HOF) calculations (Fig. 4B) under increasing shearing strains up to 1000%. HOF describes the degree of alignment (0 is random, and 1 is aligned) and was calculated from the fast Fourier transform (FFT) analysis of SEM images (10). Up to 100% strain, the traversing, randomly aligned CNTs structurally deformed into mutually aligned CNTs, and the HOF steadily increased from 0.07 to  $\sim 0.48$ . Beyond 100% strain, the HOF plateaued, showing no increase in alignment, and the intermittently contacting CNTs became increasingly bundled.

This observation at 100% strain corresponded with the measured failure strain. From these results, we propose that the strain was absorbed at low level by reversible unfolding of the traversing CNTs and beyond 100% strain by an irreversible process of straightening, slipping, and bundling of CNTs (Fig. 4C).

Transmission electron microscope (TEM) observation further revealed the intertube structure of the CNT material, which showed insight into the mechanism of energy dissipation (Fig. 4D). We found an intertube structure resembling a 3D highway network, where each CNT made contact with numerous other CNTs. This peculiar intertube structure was characterized by a high density of connections (nodes), that is, two to four CNTs intermittently contacting in parallel for only short spans ( $\sim 150$  nm). These nodes were separated mainly by isolated CNTs (struts). In addition, no CNT ends were observed, implying long, continuous CNTs, and the long CNTs were both randomly oriented and traversing. We interpret that this intertube structure of struts and nodes was the key for structural cohesiveness that allowed for large deformations. This structure differs from typically bundled CNT material (11) where CNTs are straight and contacts the same CNTs over long spans.

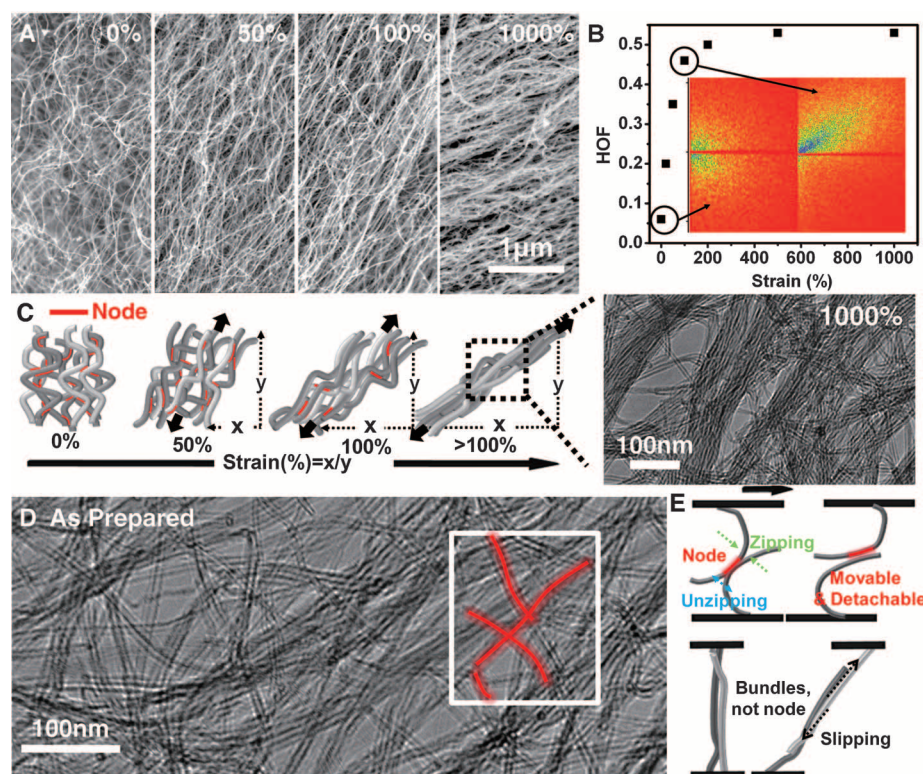
Although not directly observed, we believe that the CNTs in a node reversibly attached and

detached through zipping and unzipping (Fig. 4E). This process would dissipate energy because of the energy consumed to overcome the large van der Waals (vdW) attraction (12) between CNTs when unzipped, yet no energy is required for zipping. We interpret that sliding among CNTs was not a significant contribution because the friction coefficient (0.003) is small (13). Because the CNT material possessed a very high density of these "detachable" nodes, we interpret that they were the source of the high energy dissipation ability. Within our model, under the critical strain, the nodes perpendicular to the strain direction could reversibly zip and unzip and thereby dissipate energy. Under increased strain, the number of detachable nodes gradually decreased through either unzipping or alignment (Fig. 4C), and eventually the ability to dissipate energy decreased. Beyond the critical strain, this zipping/unzipping process was no longer reversible, and upon cycling CNTs zipped at different places and/or became bundled and aligned, resulting in degradation. A similar irreversible process caused by the slipping among CNTs under large strain has been observed in CNT yarns (11).

We estimated the loss modulus to address the validity of the zipper model. The loss modulus ( $G''$ ) of the nodes was approximated as a summation over all nodes multiplied by the energy per node to unzip and by a geometrical factor,  $\langle \cos\theta \rangle$ , to account for the fraction aligned perpendicular to the strain direction

$$G'' = \frac{E_{\text{Dissipated}}}{\gamma \dot{\gamma} \left( \frac{2\pi}{\omega} \right)} \approx \frac{1}{\gamma \dot{\gamma} \left( \frac{2\pi}{\omega} \right)} \cdot \left( \sum_N \int^l E_{\text{vdW}} dl \right) \cdot \langle \cos\theta \rangle \quad (1)$$

with vdW adhesion energy per unit length to unzip two CNTs,  $E_{\text{vdW}}$ ; node density,  $N$ ; node length,  $l = 150$  nm (by TEM, fig. S7A); shear strain and rate,  $\gamma$  and  $\dot{\gamma}$ , respectively; strain angular frequency,  $\omega$ ; and the angle between the node and the direction perpendicular to the strain,  $\theta$ . The vdW adhesion energy,  $E_{\text{vdW}}$ , was estimated as  $0.36$  nJ/m as calculated from the binding energy of two parallel cylinders following the Lennard-Jones potential (14). The CNT node density ( $4.5 \times 10^{15}$  nodes per  $\text{cm}^3$ ) was estimated by multiplying the CNT tube density ( $4.24 \times 10^{10}$  tubes per  $\text{cm}^3$ ), as estimated from the CNT bulk density ( $0.009$  g/ $\text{cm}^3$ ) and individual tube density ( $1.5 \times 10^{-13}$  g/cm), and the node density per tube ( $2.12 \times 10^4$  per tube), as estimated by the node frequency (1/300 nm) obtained by TEM images (fig. S7). With use of these values, the calculated  $G''$  was  $0.51$  MPa and agreed well with experiment ( $0.3$  MPa), which demonstrated that the energy dissipation stemmed from the unzipping vdW interaction at the nodes. In summary, the temperature invariance of the vdW interaction (15) and the thermal stability of CNT material provided this temperature invariant viscoelasticity.



**Fig. 4.** Energy dissipation model. (A) SEM images at varying shear strains. (B) Herman orientation factor (HOF) as a function of shear strain. (Inset) 2D FFT of the SEM images at 0% and 100% strain. (C) Schematic description of the change in intertube structure with strain. (Inset) TEM image of intertube structure at 1000% strain. (D) TEM image of the as-prepared intertube structure. (Inset) Selected section indicating nodes. (E) Schematic of the zipping and unzipping of nodes.

## References and Notes

- R. Lakes, *Viscoelastic Materials* (Cambridge Univ. Press, New York, ed. 1, 2009).
- E. T. Thostenson, C. Li, T. W. Chou, *Compos. Sci. Technol.* **65**, 491 (2005).
- J. Suhr *et al.*, *Nat. Nanotechnol.* **2**, 417 (2007).
- A. Cao, P. L. Dickrell, W. G. Sawyer, M. N. Ghasemi-Nejhad, P. M. Ajayan, *Science* **310**, 1307 (2005).
- X. C. Gui *et al.*, *Adv. Mater.* **22**, 617 (2010).
- Q. Zhang *et al.*, *J. Phys. D Appl. Phys.* **43**, 315401 (2010).
- S. Pathak, Z. G. Cambaz, S. R. Kalidindi, J. G. Swadener, Y. Gogotsi, *Carbon* **47**, 1969 (2009).
- K. Hata *et al.*, *Science* **306**, 1362 (2004).
- S. Osswald, E. Flahaut, H. Ye, Y. Gogotsi, *Chem. Phys. Lett.* **402**, 422 (2005).
- Materials and methods are available as supporting material on *Science* Online.
- M. Zhang, K. R. Atkinson, R. H. Baughman, *Science* **306**, 1358 (2004).
- L. Qu, L. Dai, M. Stone, Z. Xia, Z. L. Wang, *Science* **322**, 238 (2008).
- B. Bhushan, X. Ling, A. Jungen, C. Hierold, *Phys. Rev. B* **77**, 165428 (2008).
- B. Chen *et al.*, *Appl. Phys. Lett.* **83**, 3570 (2003).
- G. Lenaz, G. Milazzo, *Bioelectrochemistry of Biomacromolecules* (Birkhäuser, Basel, Switzerland, ed. 1, 1997).
- We acknowledge partial funding by TASC. M.X. acknowledges technical consultations from Y. Hayamizu, A. Izadi-Najafabadi, and Y. Seki.

## Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6009/1364/DC1  
Materials and Methods  
Figs. S1 to S9

9 July 2010; accepted 12 October 2010  
10.1126/science.1194865

# Video-Rate Molecular Imaging in Vivo with Stimulated Raman Scattering

Brian G. Saar,<sup>1\*†</sup> Christian W. Freudiger,<sup>1,2\*</sup> Jay Reichman,<sup>3</sup> C. Michael Stanley,<sup>3</sup> Gary R. Holtom,<sup>1</sup> X. Sunney Xie<sup>1‡</sup>

Optical imaging in vivo with molecular specificity is important in biomedicine because of its high spatial resolution and sensitivity compared with magnetic resonance imaging. Stimulated Raman scattering (SRS) microscopy allows highly sensitive optical imaging based on vibrational spectroscopy without adding toxic or perturbative labels. However, SRS imaging in living animals and humans has not been feasible because light cannot be collected through thick tissues, and motion-blur arises from slow imaging based on backscattered light. In this work, we enable in vivo SRS imaging by substantially enhancing the collection of the backscattered signal and increasing the imaging speed by three orders of magnitude to video rate. This approach allows label-free in vivo imaging of water, lipid, and protein in skin and mapping of penetration pathways of topically applied drugs in mice and humans.

Optical imaging techniques are complementary to magnetic resonance imaging (MRI) for in vivo applications. Although the penetration depth of MRI is much higher, optical techniques offer superior spatiotemporal resolution. Label-free optical imaging techniques for in vivo application to humans are attractive because dyes or fluorescent labels may be toxic or perturbative. Recent developments in applying two-photon autofluorescence microscopy (1) or second harmonic generation microendoscopy (2) in vivo in humans have revealed a wealth of structural and functional information that cannot be obtained with other methods, but those techniques are limited to relatively few specific molecular signatures. Raman spectroscopy offers label-free contrast based on characteristic vibrational frequencies for all major chemical species in tissue, such as lipids, water, DNA, and proteins, as well as a variety of small molecules such as drugs or metabolites. Coherent Raman scattering (3) methods allow stimulated excitation of coherent motions of vibrational oscillators and can offer vibrational imaging with subcellular spatial resolution and image acquisition speed that is more than four orders of magnitude higher than that of ordinary Raman microscopy.

Coherent anti-Stokes Raman scattering (CARS) (4, 5) has been used for fast imaging of biological samples, primarily with lipid contrast, (6, 7), at speeds up to video rate (8). However, CARS suffers from spectral distortion (9), limited sensitivity arising from an unwanted nonresonant background (10), nonlinear concentration dependence (11), and coherent image artifacts (12) that make quantitative interpretation and applications beyond lipid imaging difficult.

The recent development of stimulated Raman scattering (SRS) microscopy overcame these limitations (13–16) and provided better vibrational contrast (17, 18). In SRS microscopy, the sample is excited by colinear and tightly focused pump and Stokes beams at  $\omega_p$  and  $\omega_s$ , respectively. If the difference in frequency ( $\Delta\omega = \omega_p - \omega_s$ ) matches a molecular vibration in the sample at the frequency  $\Omega_{\text{vib}}$  (Fig. 1, A and B), the Stokes beam intensity increases and the pump beam intensity decreases as a result of the coherent excitation of molecular vibrations. When using biocompatible excitation, the intensity changes ( $\Delta I$ ) of the beams are small compared with their intensities ( $\Delta I/I < 10^{-3}$ ). We used a high-frequency modulation transfer method to detect the signal (fig. S1) (14, 19). In this approach, we modulated the intensity of the Stokes beam with an electro-optic modulator and measured the modulation transfer to the pump beam with a lock-in amplifier (LIA) after blocking the modulated Stokes beam with an optical filter. Because laser intensity fluctuations and variations in sample transmission associated with raster scanning the focus through a turbid sample occur at low frequencies, high-frequency detection

(>1 MHz) offers superior sensitivity ( $\Delta I/I < 10^{-8}$  in 1 s).

Despite the advantages of SRS contrast, it has not been applied in living animals or humans for two reasons: First, previous SRS images required ~1 min per frame, which is much too slow because living animals and humans inevitably move on the microscopic scale (movie S1). To achieve high-speed imaging (Fig. 1C), we modulated the intensity of the Stokes beam at 20 MHz and used a home-built, all-analog lock-in amplifier with a response time of ~100 ns (fig. S2). The colinear laser beams were tuned to match a vibrational frequency of interest (Fig. 1B) and raster-scanned across the sample by a resonant galvanometer mirror with a line rate of 8 kHz (100 ns per pixel at  $512 \times 512$  pixels with up to 25 frames/s). The power levels used here were previously used for in vivo imaging, and no photodamage was observed (8). Whereas previous SRS microscopy was limited by the 100- $\mu$ s response time of a commercial lock-in amplifier (SR844, Stanford Research Systems), our new system is three orders of magnitude faster.

Second, SRS microscopy involves measurement of the intensity loss of the transmitted pump beam, which is not feasible in thick, nontransparent samples (for instance, a human arm). We must rely on backscattering of the forward-going signal by tissue. This signal was previously collected by the excitation objective (14), which works for samples with extremely high scattering (18). However, in biological tissues, the epi-directed signal collected through the objective is too weak for high-speed imaging: Even with >60-s integration time, the signal-to-noise ratio obtained from tissue was poor (14).

To quantitatively understand the light collection in epi-SRS, we performed nonsequential ray-tracing simulations (20) by simulating the propagation of a large number ( $10^6$ ) of rays emitted from the focal volume into a bulk medium with user-defined scattering parameters, which is used as a model for tissue (Fig. 1, D and E). Scattering events along a ray's trajectory are modeled probabilistically (21), and we measured the final distribution of the light after it is emitted from the simulated tissue volume. We found that, as expected, 40 to 45% of the light is backscattered in a thick tissue sample because scattering dominates over absorption in most tissues (22). Because of multiple scattering, the diffuse cloud of backscattered light has a radius of ~5 mm at the front aperture of the objective lens (Fig. 1F), largely independent of the excitation numerical

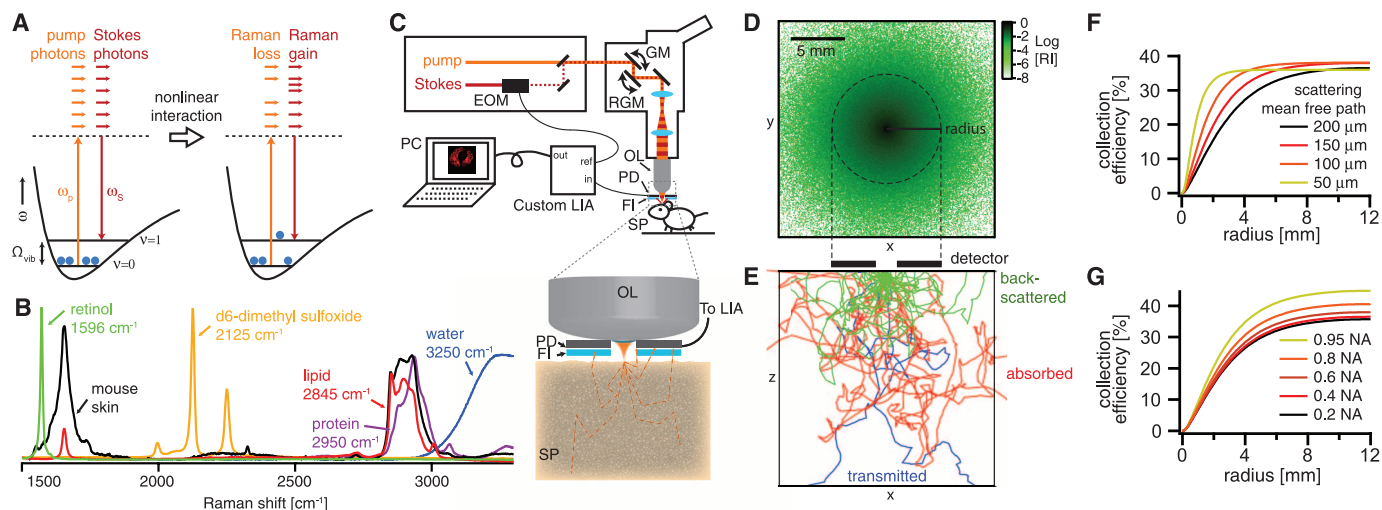
<sup>1</sup>Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138, USA. <sup>2</sup>Department of Physics, Harvard University, Cambridge, MA 02138, USA. <sup>3</sup>Chroma Technology, 10 Imtec Lane, Bellows Falls, VT 05101, USA.

\*These authors contributed equally to this work.

†Present address: MIT Lincoln Laboratory, 244 Wood Street, Lexington, MA 02420, USA.

‡To whom correspondence should be addressed. E-mail: xie@chemistry.harvard.edu





**Fig. 1. SRS microscopy.** (A) Energy diagram of SRS. If the difference frequency of the excitation beams ( $\Delta\omega = \omega_p - \omega_s$ ) matches a vibrational frequency in the sample,  $\Omega_{\text{vib}}$ , a molecule is excited from the vibrational ground state ( $v = 0$ ) to a vibrational excited state ( $v = 1$ ), passing through a virtual state (dashed lines). This results in a photon in the pump field being annihilated (stimulated Raman loss) and a photon in the Stokes field being created (stimulated Raman gain), which can be probed as a contrast for microscopy. (B) Raman spectra of chemical compounds imaged in this work. (C) Our experimental setup for in vivo SRS microscopy detected the modulation transfer from the Stokes beam to the backscattered pump beam. (Inset) The epi-detector assembly. The specimen (SP) is excited by focusing light with an objective lens (OL) through a small hole in the center of the large-area epi-detector (PD) with a 10-mm outer diameter and a 2-mm aperture in the center. Multiple scattering within the tissue sample redirects a large portion of the forward-

traveling light to illuminate the detector active area. The modulated Stokes beam is blocked by an optical filter (FI), and the transmitted pump beam is detected and demodulated by the LIA. Images are constructed by scanning the focal spot in two dimensions with the use of a galvanometer mirror (GM) and resonant galvanometer mirror (RGM). EOM, electro-optic modulator. (D) Ray-tracing simulations allow quantitative understanding of the distribution of the relative intensity (RI) of the backscattered light at the tissue surface from a focus at a depth of 100  $\mu\text{m}$  into the tissue (scattering mean free path = 200  $\mu\text{m}$ , anisotropy = 0.9) emitting in the forward direction with a 0.4 numerical aperture. (E) Depth profile of tissue showing sample ray trajectories colored according to one of three final outcomes. (F and G) Simulations of collection efficiency of backscattered, forward-traveling light versus detector radius for (F) different scattering mean free paths of the sample and (G) different numerical apertures (NA) of the excitation objective.

aperture (Fig. 1G). A typical microscope objective has a front-aperture radius of  $\sim 1$  to 2 mm, so more than 90% of the backscattered light is not collected by the objective, unless it is surrounded by specially-designed parabolic collection mirrors (23). We solved this problem by placing the photodetector directly in front of the objective lens and exciting through an aperture in the center of the detector (Fig. 1C). In this geometry, we found in mouse skin that we collected  $\sim 28\%$  of the laser light impinging onto the sample. Given the angular distribution of the backscattered light (fig. S3), a filter to block the modulated Stokes beam while transmitting the pump beam had to be specially designed (fig. S4).

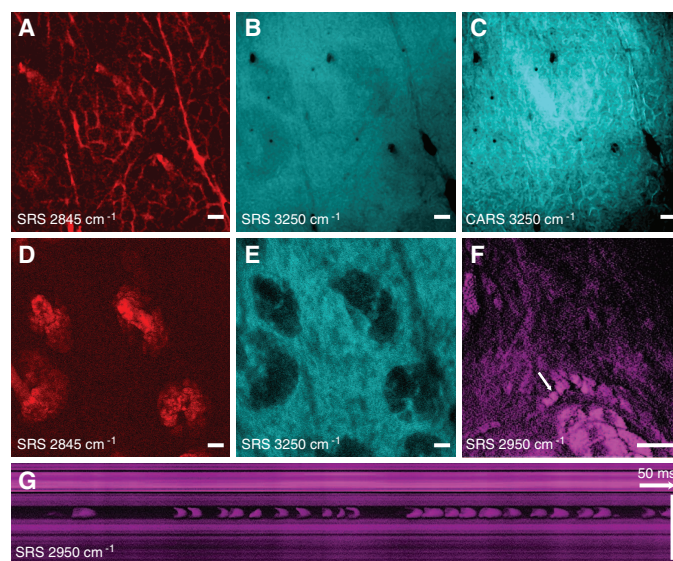
Using this system, we imaged skin in vivo in mice. Figure 2 shows single SRS video-rate frames obtained by the  $\text{CH}_2$  stretching (primarily lipids; Fig. 2, A and D), OH stretching (primarily water; Fig. 2, B and E), and  $\text{CH}_3$  stretching (primarily protein; Fig. 2, F and G) vibrations. The lipid distributions (movie S2) are as expected from previous work (14), but water can only be measured in vivo because the skin hydration changes in excised tissue. Imaging water is of particular interest in studying the transport properties of water-soluble drugs and their effect on the hydration of the skin barrier (24).

Figure 2C highlights that CARS imaging of water is distorted by the nonresonant background, which introduces an image artifact. It shows a positive contrast “honeycomb” pattern for the lipid-rich areas of the stratum corneum layer, which do not contain water. Thus, the contrast in CARS is inverted compared with the real water distribution.

**Fig. 2. SRS skin imaging in living mice.** (A) SRS image of lipids of the stratum corneum shows intercellular spaces between hexagonal corneocytes; (B) SRS water image ( $3250\text{ cm}^{-1}$ ) of the same region shows a homogeneous distribution of water. (C) CARS water image acquired simultaneously with (B) shows artifacts from the nonresonant background of lipids. (D) SRS lipid and (E) water images of the viable epidermis show sebaceous glands with positive and negative contrast, respectively. (F) SRS images of the viable epidermis at the  $\text{CH}_3$  stretching vibration ( $2950\text{ cm}^{-1}$ ) mainly highlight proteins, as well as residual lipid-rich structures. A capillary with individual red blood cells (arrow) is visible. The cells are imaged without motion blur due to video-rate acquisition speed. (G) SRS in vivo flow cytometry. An x-t plot acquired by line-scanning across a capillary at the position of the arrow in (F) is shown. Individual red blood cells are captured on the fly. Images in (A) to (E) are acquired in epi-direction, whereas those in (F) and (G) are acquired in transmission, all with 37-ms-per-frame acquisition speed and  $512 \times 512$  pixel sampling. Scale bars, 25  $\mu\text{m}$ . The Raman spectra are shown in Fig. 1B.

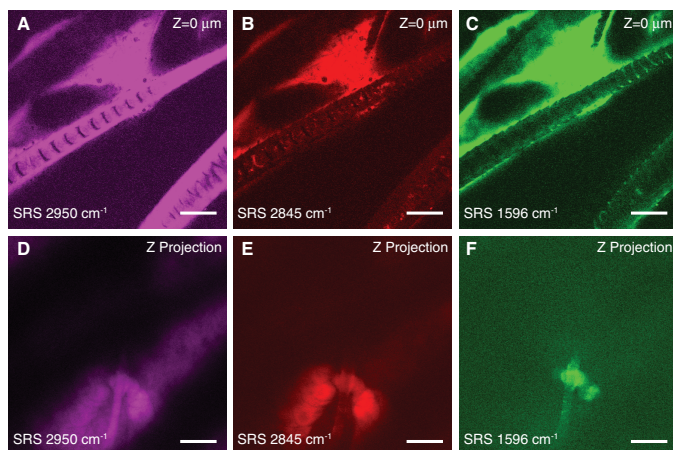
This effect is not observed in SRS because it is free from this nonresonant background (14). Signal averaging can further improve the signal-to-noise ratio if the sample remains still enough (fig. S5).

The protein images (Fig. 2, F and G, and fig. S6) show red blood cells moving in a capillary of



the viable epidermis (movie S3), due primarily to the contrast of  $\text{CH}_3$  oscillators in hemoglobin. By performing line scans versus time, we reconstructed a plot of the cells as they pass through the scan line (Fig. 2G), allowing for in vivo flow cytometry (25) based on intrinsic chemical contrast.

**Fig. 3.** In vivo imaging of drug penetration of *trans*-retinol. (A to C) SRS images of hair and stratum corneum in the ear of a living mouse. (D to F) Depth projection of 3D image stacks of the viable epidermis. Images are acquired at the indicated Raman shifts. Contrast coming primarily from protein ( $2950\text{ cm}^{-1}$ ) and lipid ( $2845\text{ cm}^{-1}$ ) shows the morphology of the skin with all its structural elements and sub-cellular resolution [see nuclei in (E)]. Nuclei are visible as dark structures in the  $2845\text{ cm}^{-1}$  image (E), because the nuclei have low concentrations of lipids. Hairs are visible as solid, periodic structures in the protein image (D) and are surrounded by oil secreted from the sebaceous glands in the lipid image (E). We are able to visualize with SRS that drug penetration of the topically applied *trans*-retinol (C) occurs along the hair shaft (F). Images are collected in transmission with 37-ms-per-frame acquisition speed and  $512 \times 512$  pixel sampling. Scale bars,  $25\text{ }\mu\text{m}$ . The Raman spectra are shown in Fig. 1B.



**Fig. 4.** SRS skin imaging in living humans. (A to C) SRS images of the stratum corneum and viable epidermis tuned into  $\text{CH}_3$  stretching vibration of proteins ( $2950\text{ cm}^{-1}$ ) showing the stratum corneum (A), the viable epidermis (B), and a hair on the skin surface (C). (D) SRS image of DMSO penetrating the skin at the same region as shown in (C). We find that DMSO also accumulates in the hair shaft. We used deuterium labeling to create a unique vibration of  $\text{d}_6$ -DMSO at  $2120\text{ cm}^{-1}$  for specific imaging. Images are acquired in epi-direction on the forearm of a volunteer (X.S.X.). Image acquisition time is 150 ms for (A) and (B) and 37 ms for (C) and (D), all with  $512 \times 512$  pixel sampling. Scale bars,  $50\text{ }\mu\text{m}$ . The Raman spectra are shown in Fig. 1B.

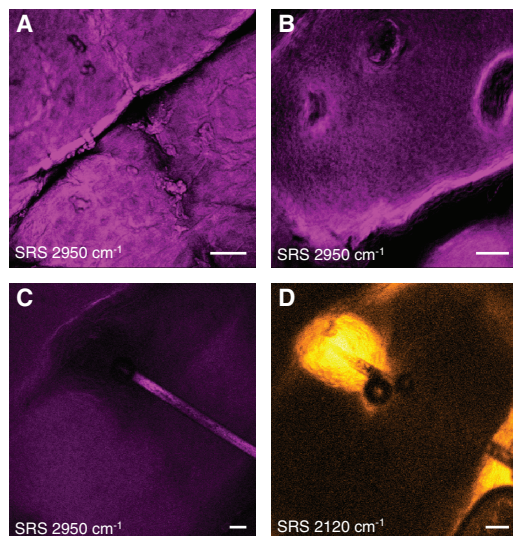


Figure 3 demonstrates the ability of SRS microscopy to follow the penetration of a small-molecule drug (*trans*-retinol, which stimulates collagen synthesis) into mouse skin. Contrast using the  $\text{CH}_3$  and  $\text{CH}_2$  stretching highlights the morphology of the skin. By tuning in to the polyene stretch of *trans*-retinol, we see that the drug has penetrated via the hair shaft into the sebaceous gland, one of three hypothesized penetration routes into skin (26, 27). Averaging the three-dimensional (3D) data (movie S4), we see the localization of *trans*-retinol surrounding the hair and in the top of the sebaceous gland (Fig. 3F). In our previous work, performed excised tissue, we did not observe this pathway for this drug (14), indicating the importance of in vivo studies, because the transport properties of small molecules can be affected by the skin temperature, moisture content, and other factors.

Figure 4 shows in vivo SRS imaging of human skin. Cell layers of the viable epidermis up to a

depth of  $50\text{ }\mu\text{m}$  can be seen when tuned in to the  $\text{CH}_3$  stretching band. High contrast is available for nuclei (Fig. 4, A and B), and the varying nuclear size can be used to find the boundary between the viable epidermis and the stratum corneum. Figure 4C shows a hair disappearing down the hair shaft. In this case, deuterated dimethyl sulfoxide (DMSO), a penetration-enhancing small molecule, was applied to the skin. By tuning to the C-D stretching vibration at  $2125\text{ cm}^{-1}$ , contrast from DMSO appeared. DMSO can again be found to accumulate in the area surrounding the hair, though it does not completely penetrate into the hair itself. Such mechanistic insight into the transport of small molecules can only be obtained with label-free chemical imaging.

By overcoming the challenges associated with performing high-speed, epi-detected SRS microscopy, this powerful label-free imaging modality can now be applied to a broad range of problems in whole living organisms, including

small animals and humans. The approach presented here can also be applied to other modulation transfer imaging techniques, including two-color two-photon absorption, pump-probe (28), and stimulated emission (29) microscopy. High-speed vibrational imaging is likely to play an increasingly important role in medical diagnostics in humans.

## References and Notes

- K. König *et al.*, *Microsc. Res. Tech.* **70**, 398 (2007).
- M. E. Llewellyn, R. P. J. Barretto, S. L. Delp, M. J. Schnitzer, *Nature* **454**, 784 (2008).
- M. D. Levenson, S. S. Kano, *Introduction to Nonlinear Laser Spectroscopy* (Academic Press, San Diego, 1988).
- A. Zumbusch, G. R. Holtom, X. S. Xie, *Phys. Rev. Lett.* **82**, 4142 (1999).
- C. L. Evans, X. S. Xie, *Annu. Rev. Anal. Chem.* **1**, 883 (2008).
- T. B. Huff, J. X. Cheng, *J. Microsc.* **225**, 175 (2007).
- T. Hellerer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 14658 (2007).
- C. L. Evans *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 16807 (2005).
- H. A. Rinia, M. Bonn, M. Müller, *J. Phys. Chem. B* **110**, 4472 (2006).
- F. Ganikhanov, C. L. Evans, B. G. Saar, X. S. Xie, *Opt. Lett.* **31**, 1872 (2006).
- L. Li, H. Wang, J.-X. Cheng, *Biophys. J.* **89**, 3480 (2005).
- J. X. Cheng, A. Volkmer, X. S. Xie, *J. Opt. Soc. Am. B* **19**, 1363 (2002).
- E. Ploetz, S. Laimgruber, S. Berner, W. Zinth, P. Gilch, *Appl. Phys. B* **87**, 389 (2007).
- C. W. Freudiger *et al.*, *Science* **322**, 1857 (2008).
- Y. Ozeki, F. Dake, S. Kajiyama, K. Fukui, K. Itoh, *Opt. Exp.* **17**, 3651 (2009).
- P. Nandakumar, A. Kovalev, A. Volkmer, *New J. Phys.* **11**, 033026 (2009).
- B. G. Saar *et al.*, *Angew. Chem. Int. Ed.* **49**, 5476 (2010).
- M. N. Slipchenko *et al.*, *Analyst (London)* **135**, 2613 (2010).
- Methods, additional results, and movies are available as supporting material on Science Online.
- Zemax User's Guide (Zemax Development Corporation, Bellevue, WA, 2010).
- S. L. Jacques, C. A. Alter, S. A. Pahl, *Lasers Life Sci.* **1**, 309 (1987).
- W. F. Cheong, S. A. Pahl, A. J. Welch, *IEEE J. Quant. Elec.* **26**, 2166 (1990).
- C. Combs *et al.*, *J. Microsc.* **228**, 330 (2007).
- A. V. Rawlings, C. R. Harding, *Dermatol. Ther.* **17** (suppl. 1), 43 (2004).
- D. A. Sipkins *et al.*, *Nature* **435**, 969 (2005).
- M. R. Prausnitz, S. Mitragotri, R. Langer, *Nat. Rev. Drug Discov.* **3**, 115 (2004).
- J. Lademann *et al.*, *Skin Pharm. Phys.* **21**, 150 (2008).
- D. Fu, T. Ye, T. Matthews, G. Yurtsever, W. Warren, *J. Biomed. Opt.* **12**, 054004 (2007).
- W. Min *et al.*, *Nature* **461**, 1105 (2009).
- We thank J. MacArthur for advice on electronics and K. Sherwood, W. Faustino, and X. Zhang for helpful discussions. C.W.F. was supported by a Boehringer Ingelheim Fonds Ph.D. fellowship. This work was supported by the Bill and Melinda Gates Foundation and an NIH T-R01 award (1R01EB010244-01) to X.S.X. Patent applications based on this work have been filed by Harvard Univ. Imaging was performed in accordance with Harvard Univ. Faculty of Arts and Sciences Institutional Animal Care and Use Committee protocol number 29-01.

## Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6009/1370/DC1  
Methods

Figs. S1 to S6

Movies S1 to S4

1 September 2010; accepted 4 November 2010  
10.1126/science.1197236



# The Role of Particle Morphology in Interfacial Energy Transfer in CdSe/CdS Heterostructure Nanocrystals

Nicholas J. Borys,<sup>1</sup> Manfred J. Walter,<sup>1</sup> Jing Huang,<sup>2</sup> Dmitri V. Talapin,<sup>2,3</sup> John M. Lupton<sup>1,4\*</sup>

Nanoscale semiconductor heterostructures such as tetrapods can be used to mimic light-harvesting processes. We used single-particle light-harvesting action spectroscopy to probe the impact of particle morphology on energy transfer and carrier relaxation across a heterojunction. The generic form of an action spectrum [in our experiments, photoluminescence excitation (PLE) under absorption in CdS and emission from CdSe in nanocrystal tetrapods, rods, and spheres] was controlled by the physical shape and resulting morphological variation in the quantum confinement parameters of the nanoparticle. A correlation between single-particle PLE and physical shape as determined by scanning electron microscopy was demonstrated. Such an analysis links local structural non-uniformities such as CdS bulbs forming around the CdSe core in CdSe/CdS nanorods to a lower probability of manifesting excitation energy-dependent emission spectra, which in turn is probably related to band alignment and electron delocalization at the heterojunction interface.

Advances in the synthesis of semiconductor nanoparticles have enabled exquisite control over composition and shape, yielding spherical, linear (1, 2), and branched structures such as tetrapods (3–5). Although many semiconductor nanoparticles originally consisted of only one material, further opportunities for tailoring electronic functionalities are anticipated from nanoscale semiconductor heterostructures combining two or more materials (5). Indeed, a host of applications has emerged from precise control over nanostructure functionality, ranging from photovoltaics (2, 6) to commercial light-emitting devices. A microscopic understanding of the migration of excitation energy in heterojunctions and the resulting interfacial charge transfer (7) is particularly important in developing photocatalytic compounds to split water (8) or reduce CO<sub>2</sub> (9). Due to only a slight mismatch in the lattice constants of the two materials, yet well-contrasted band gaps, the CdSe/CdS heterostructure has evolved as one of the workhorses for relating nanoparticle synthesis and shape to spectroscopic properties. Here we describe an optical classification of the quantum confinement in complex CdSe/CdS core/shell nanostructures by means of single-particle light-harvesting action spectroscopy, a versatile noncontact tool to measure electron transport or energy transfer on mesoscopic length scales (10). For a variety of nanostructure shapes, we probed the photoluminescence excitation (PLE) of the absorbing CdS through emission from the lower-gap CdSe. We identified two distinct categories of action spectra, characteristic

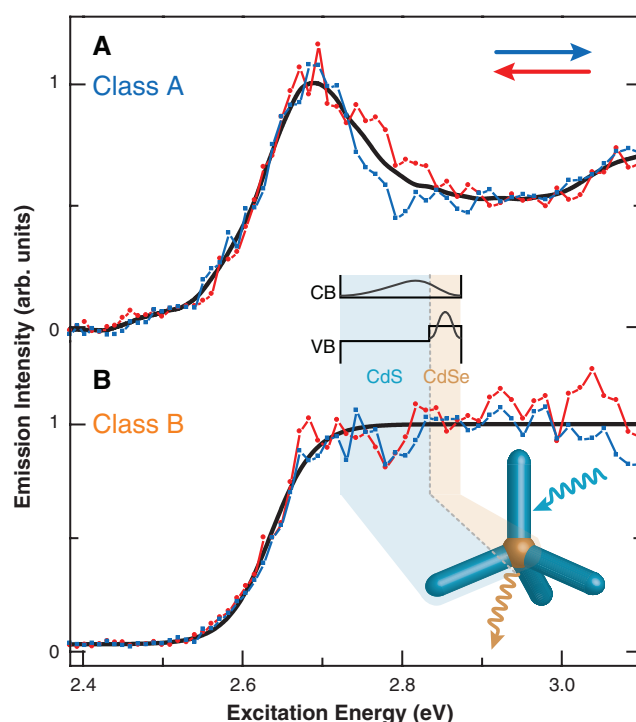
of the physical shape and quantum confinement parameters of the semiconductor heterostructures, and showed how the energetic landscape can inhibit complete electron transfer across the CdSe/CdS interface. This single-particle classification highlights the sensitivity of ensemble performance, such as in light-harvesting, to morphological characteristics that affect the intrinsic nature of the interfaces, thereby suggesting routes to future synthetic optimization.

Large optical absorption cross sections, enhanced stability, high quantum yields, and size-tunable electronic structure make semiconductor nanocrystals particularly interesting for light-

harvesting and energy conversion applications (2, 6). Nevertheless, there is still no clear consensus on the microscopic nature of interfacial energy levels and the process of carrier migration from one semiconductor to another. Very similar CdSe/CdS nanostructures can exhibit properties of either a type I heterostructure, in which both electron and positively charged hole are confined to one material, or a type II heterostructure, in which electron and hole separate between the materials (11–14). Naturally, such a distinction is crucial to choosing the correct material for a particular application: Light emission requires type I junctions, whereas charge separation in photovoltaics requires type II (13). Such knowledge of the interface is crucial for designing nanoparticle superstructures, such as inorganic dendrimers with a treelike branched architecture, with superior light-harvesting properties. A first step to such a structure is the heterojunction tetrapod (5).

Semiconductor nanoparticles are well suited to microscopic optical studies, which have revealed a surprising diversity in electronic properties between particles that are typically masked in ensemble-level measurements (15). Htoon *et al.*, for example, uncovered both discrete excitonic states and a quasi-continuum in the core of spherical CdSe particles capped with a ZnS shell (16). We probed energetic relaxation in individual CdSe/CdS nanoheterostructures of different shapes after optical excitation of the higher-gap CdS. This approach, leveraging single-particle PLE over a wide spectral range, illuminates the nature of both the high-gap material absorption and the subsequent intraparticle relaxation pro-

**Fig. 1.** Light-harvesting action spectroscopy at 4 K of two single semiconductor tetrapods consisting of a low-gap CdSe core and high-gap CdS arms. The nanoparticles are excited primarily above the CdS absorption edge, and emission is detected from the CdSe core. A sketch of the assumed electron and hole probability density distribution within the electronic band structure is shown in the inset in the middle. (A) For the first particle, the onset of absorption from the quantum-confined CdS exciton gives rise to a distinct peak that is then followed by absorption into a continuum of states at higher energy (red, scan downward in energy; blue, scan upward). (B) For the second particle, the peak associated with the CdS exciton is not present, and the spectrum is marked by a continuum of states after the onset of CdS absorption (the black lines are a guide to the eye).



<sup>1</sup>Department of Physics and Astronomy, University of Utah, Salt Lake City, UT 84112-0830, USA. <sup>2</sup>Department of Chemistry, University of Chicago, Chicago, IL 60637, USA. <sup>3</sup>Center for Nanoscale Materials, Argonne National Laboratory, Argonne, IL 60439, USA. <sup>4</sup>Institut für Experimentelle und Angewandte Physik, Universität Regensburg, D-93040 Regensburg, Germany.

\*To whom correspondence should be addressed. E-mail: john.lupton@physik.uni-regensburg.de

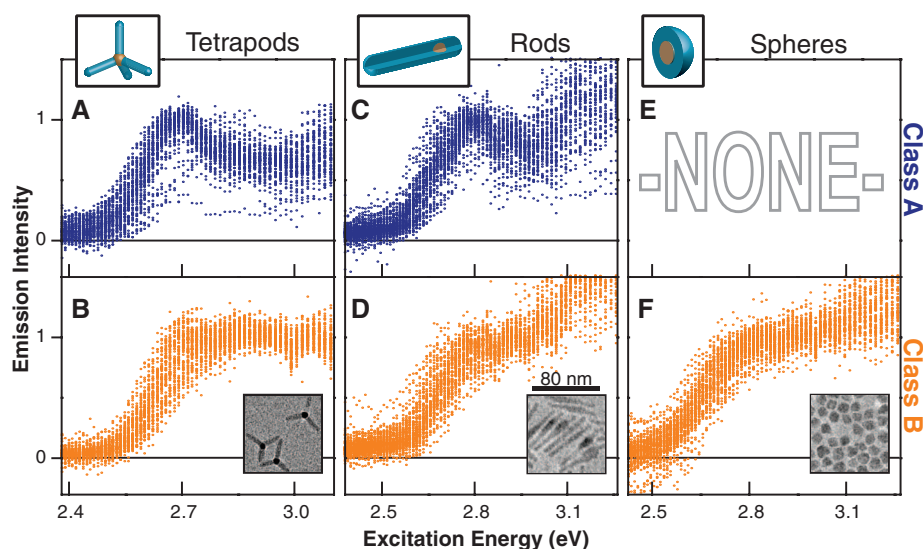
cess, which are both ultimately crucial in designing light-harvesting applications (6). In contrast, most single-quantum-structure PLE spectroscopy (16–18) to date has been carried out over relatively narrow spectral ranges to probe distinct excitonic features, or else has analyzed excitation enhancement by metal nanoparticles at discrete wavelengths (19).

We focused our attention on CdSe/CdS tetrapods, which are inorganic light-harvesting complexes with very large absorption cross sections, which make them easily visible in single-particle measurements. A schematic of a CdSe/CdS tetrapod nanoparticle and a suggestive band diagram (20) are shown in the inset of Fig. 1. The structures consist of a CdSe core approximately 4 nm in diameter, which is surrounded by an antenna-like CdS shell. The shell has four arms that are 20 nm in length and 6 nm in diameter. We purified the tetrapod solution to an ensemble composition of at least 90% tetrapods, which appeared to be geometrically uniform in transmission electron microscopy (TEM) (5, 21). Previous comparisons of theory and experiment have indicated that, on average, the CdSe/CdS interface should form a quasi-type II heterostructure, with an excited electron delocalizing over the CdSe and CdS conduction bands and the associated hole localizing on the lower-gap CdSe core (14). Initial studies on the tetrapods have suggested a similar electronic structure (20), although the energy offsets are such that a range of interfacial localization phenomena is conceivable (13): It is not straightforward to extrapolate band diagrams derived from bulk material parameters to solution-grown nanostructures. For these heterostructures, at energies above the CdS fundamental absorption edge, the CdS extinction dwarfs that of the CdSe, thus resulting in an absorption spectrum dominated by the CdS. The electronic structure, on the other hand, yields a luminescence spectrum arising from recombination in CdSe (5, 21). The similarity of the ensemble nanoparticle PLE and absorption spectra indicates that this light-harvesting process is highly efficient (5). We expect the prominent onset of the PLE to correspond with the quantum-confined exciton of the CdS, followed by the typical continuum of higher energy states.

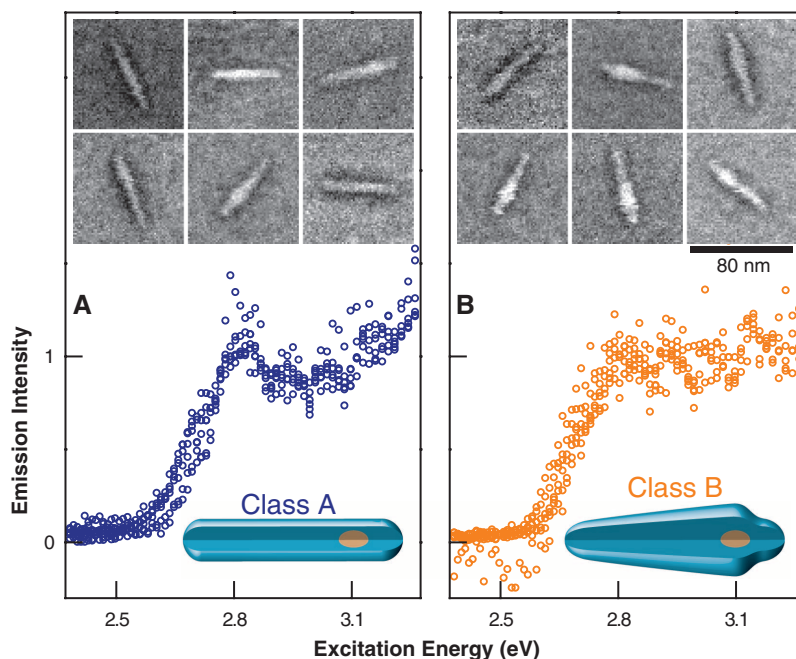
Such behavior was indeed observed in single-particle light-harvesting action at 4 K in Fig. 1A. We recorded the CdSe emission intensity while sweeping the laser excitation both down and up in energy (22). The particle exhibited a clear peak in the CdS absorption that can be attributed to the quantum-confined excitonic transition (23, 24). We refer to PLE spectra with such behavior as class A. A second single tetrapod showed strikingly different behavior depicted in Fig. 1B, revealing particle-to-particle variations that are masked in ensemble measurements. In this case, there is no clear peak after the CdS absorption onset. We term these PLE spectra class B. Both classes were repeatedly observed in spatial proximity during the same excitation scan.

Although the tetrapods perform the desired light-harvesting function, they do not offer the best system in which to search for structural ori-

gins of the distinct PLE classification. We therefore used two further model systems to study the influence of particle form on PLE: nanorods,



**Fig. 2.** Classification of single-particle light-harvesting action spectra for tetrapod, rod, and near-spherical nanoheterostructures. A total of 150, 150, and 75 raw spectra for tetrapods, rods, and spheres, respectively, are sorted into two groups. All curves are normalized to the CdS absorption onset intensity but are otherwise not manipulated. (A and B) 75 class A and 75 class B single-particle PLE spectra from tetrapod structures. (C and D) 75 class A and 75 class B single-particle PLE spectra from rod structures. (E and F) The near-spherical structures show only class B single-particle PLE spectra. Inset in (B) to (F) are representative TEM images of the tetrapods, rods, and spheres. Although the tetrapods appear uniform, the rod ensemble manifests two morphologies: one with a uniform diameter and the other with a pronounced bulb feature. The near-spherical structures are non-uniform in their deviations from isotropy.



**Fig. 3.** Correlated SEM and PLE spectra of 12 single CdSe/CdS nanorods. (A) Six class A PLE spectra normalized to the CdS onset and plotted together with their corresponding SEM images, representative of rods with a uniform diameter (cartoon at bottom). (B) The SEM images of six rods that exhibit a class B PLE spectrum indicate that the spectral shape is attributable to rods that have a pronounced bulb feature and non-uniform diameter as sketched schematically in the cartoon. All correlated image-PLE pairs were chosen solely on the basis of the quality of the PLE spectrum, without any knowledge of the corresponding SEM image.



which mimic one arm and the core of the tetrapod; and nanospheres with a thick CdS shell. All structures had a similar CdSe core that was 4 nm in diameter. Figure 2 shows single-particle spectra of 150 tetrapod, 150 rod, and 75 near-spherical nanoheterostructures, normalized to the CdS absorption onset. Representative TEM images for each nanostructure are shown in the lower insets. The rods consist of a CdS arm 60 nm in length and approximately 6 nm in diameter surrounding the CdSe core. TEM indicated that the sample contained two general shapes of rods: rods that have a constant diameter over their length and those that have a bulb feature surrounding the CdSe core, resulting in a non-uniform rod diameter (11, 21). Finally, the CdS shell for the near-spherical structures was a 4-nm-thick layer surrounding the CdSe core. TEM of these particles revealed that the thick CdS shell results in a non-uniform distribution of particles with anisotropic deviations from a perfect sphere. For the tetrapods and rods, each single-particle PLE spectrum can generally be categorized as class A (Fig. 2, A and C, respectively) or class B (Fig. 2, B and D, respectively). In contrast, we observed exclusively class B spectra for the near-spherical structures (Fig. 2F). We conclude that the origin of the different classes is not found in the combination of constituent materials but is related to the shape of the nanocrystal, which de-

termines the symmetry of the confinement potential. Other approaches to representing the raw data, along with quantitative thresholds for differentiating between the two classes, are discussed in figs. S3 and S4 (22).

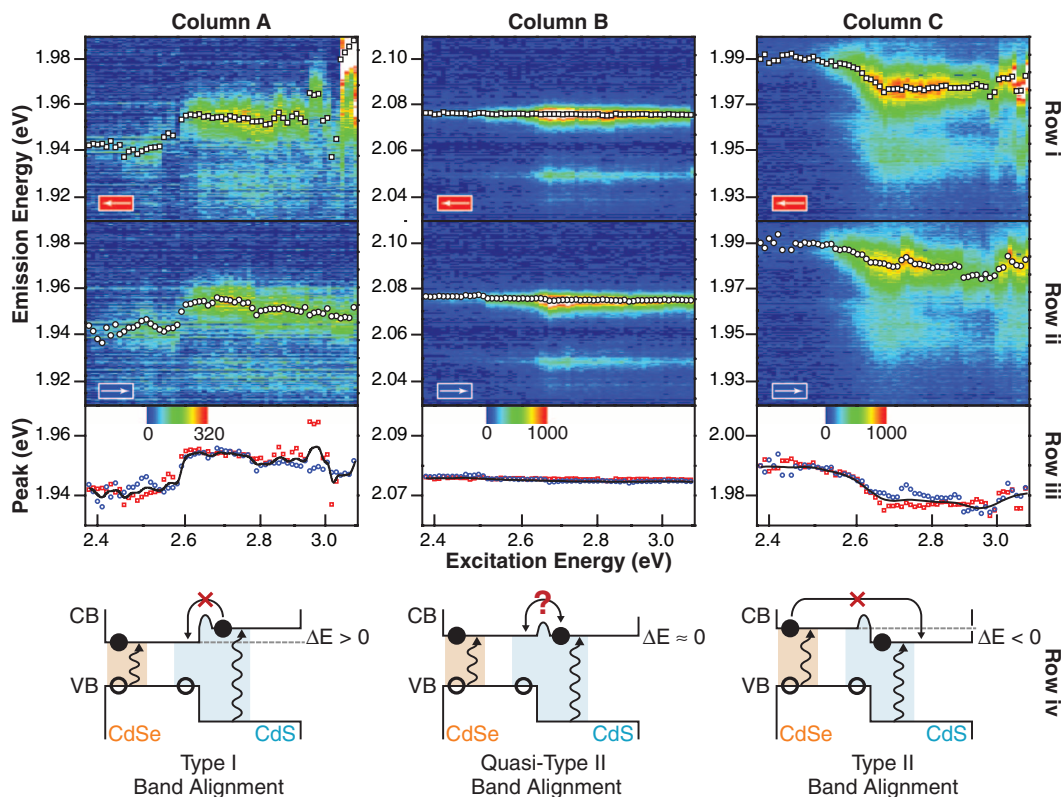
Through its relation to the quantum confinement effect, physical shape plays a crucial role in the optical and electronic properties of semiconductor nanocrystals (13, 23, 25). For the rods, where quantum confinement in the CdS is influenced mostly by diameter (26), the bulb structures consist of a range of diameters that will smear out the excitonic transition and will likely lead to particles with a class B spectrum. The same effect of shape variations in the direction of radial quantum confinement could also give rise to exclusively class B spectra in the near-spherical particles. In addition, quantum confinement in absorption in these particles may be weaker as the larger particle diameters approach the Bohr exciton radius in CdS, thus reducing the visibility of excitonic transitions.

To verify the role that shape plays in the PLE spectrum of a single nanocrystal, we correlated PLE measurements with scanning electron microscopy (SEM) of single rods. The correlation process is discussed in the text accompanying figs. S6 and S7. Figure 3 shows 12 PLE spectra and the SEM images of the corresponding nanorods. The pairs were selected solely on the

basis of the quality of the PLE spectrum over up and down sweeps without any knowledge of the corresponding image. Figure 3A shows six class A PLE spectra normalized and plotted together; Fig. 3B shows six class B spectra. Inset in the plot are the corresponding SEM images. A clear trend that confirms the shape hypothesis is observable: All of the rods in Fig. 3A exhibit a class A PLE spectrum, and five out of six have a uniform diameter over the length of the rod. On the other hand, in Fig. 3B, all of the rods exhibit a class B PLE spectrum and have a pronounced bulb feature as anticipated (see the text accompanying fig. S7 for a discussion of the geometric thresholds invoked). These observations are consistent with single-particle PL (fig. S8), which demonstrates that rods with a class A PLE spectrum have higher-energy residual CdS emission due to their smaller diameters (stronger quantum confinement) than rods with a class B PLE spectrum. The basic statistical analysis presented in table S1 indicates that the probability of the results in Fig. 3 occurring by chance is 0.3%.

The similarity in PLE and emission spectra of rods, spheres, and tetrapods suggests that excessive CdS growth around the core could be responsible for the formation of two distinct spectroscopic classes of tetrapods. In addition, variations in effective arm diameter within a single tetrapod (20) or, quite generally, variations in

**Fig. 4.** Single-particle emission spectra as a function of excitation energy for three individual class A tetrapods, revealing the possible band alignment schemes. (**Column A**) The emission spectrum shifts to higher energies as the excitation energy is raised. Two-dimensional plots of emission as a function of excitation are shown for the laser sweep downward (Row i) and upward (Row ii) in energy. The main emission peak energy, extracted by fitting two Gaussians to the emission spectrum, is overlaid as white circles. (Row iii) The peak position as a function of excitation energy shows a steplike shift of  $\sim 10$  meV close to the absorption onset of CdS at 2.6 eV. (Row iv) This situation can be rationalized in terms of a type I band alignment, where a barrier to thermalization of the electron from the CdS to the CdSe exists. Below 2.6 eV, direct excitation of the CdSe core occurs, whereas emission at higher excitation energies results from an interfacial exciton. (**Column B**) No spectral shift is seen with excitation energy, suggesting a quasi-type II band alignment. By analogy to column A, it is conceivable that a barrier to electron transfer between CdS and CdSe also exists due to, for example, lattice strain. (**Column C**) The type II band alignment is characterized by a shift of emission to lower energies as the excitation energy is raised from absorption in



the CdSe core to the CdS shell. A barrier to electron transfer from CdSe to CdS must exist to prevent the core electron from transferring to the shell under excitation of the core. The black lines in the peak position plots are smoothed averages that serve as a guide for the eye.

intraparticle CdS–CdSe coupling strength that disrupt the particle symmetry, could lead to a loss of excitonic structure in PLE. We therefore propose that the single-particle PLE can serve as a predictor of particle morphology and indeed provides insight into the tetrapod shape, which is hard to obtain by other means.

Bulblike particle morphologies probably have featureless PLE spectra because the CdS shell is not defined by one fixed diameter, and therefore a range of excitonic transition energies exists. This morphological disorder within a single particle also appears to affect band alignment within the heterojunction, as revealed by spectrally resolving tetrapod emission as a function of excitation energy for 50 single tetrapods. Of these tetrapods, 26% [13 tetrapods (fig. S9)] showed a surprising dependence of the tetrapod emission energy on the excitation energy. Three such cases are detailed in Fig. 4.

The particle in column A of Fig. 4 exhibits a shift in emission to higher energy as the excitation energy is raised, although the shape of the emission spectrum remains characterized by the CdSe electronic transition and its phonon sideband. It is important to consider both sweep directions of the exciting laser because nanocrystals exhibit random spectral diffusion (27); the spectral changes are reversible upon downward (row i) and upward (row ii) sweeps. Row iii of column A plots the PL peak energy, which shows a distinct jump at an excitation energy of 2.6 eV. The position of the spectral jump corresponds to the onset of CdS absorption at 4 K and indicates a transition from direct excitation of the CdSe core to excitation of the CdS arm and subsequent relaxation to the CdSe. In contrast, the particle in column B exhibits emission independent of excitation. This case would be expected for a semiconductor heterostructure in which the high-energy electron-hole pair formed in the wide-gap semiconductor (CdS) relaxes down to the narrower-gap CdSe. Finally, in column C, the emission energy decreases with increasing excitation energy. As in column A, a change in the emission peak is observed around excitation at 2.6 eV.

To rationalize these observations, we consider the subtleties of conduction and valence band offsets between CdSe and CdS in row iv of Fig. 4. Studies of CdSe/CdS nanoparticles at the ensemble level have suggested that the CdSe valence band is consistently higher in energy than that of the CdS, although the conduction band alignment of the two materials is sensitive to nanoparticle geometry and may result in a type I, type II, or quasi-type II electronic structure (see sketches in Fig. 4 for definition) (11–13). Such band offsets will affect the emission spectrum if a barrier in the conduction band inhibits electron transfer between CdS and CdSe, which is conceivable given the interfacial strain due to the lattice mismatch of the two materials (28). We propose that the tetrapods in columns A and C of Fig. 4 are characterized by conduction band

offsets  $\Delta E$ , so that at least two distinct exciton states exist: CdSe core excitons and CdSe/CdS interfacial excitons. Because both states are emissive and hence stable, an interfacial barrier must be present, which prevents complete electron transfer to the lowest state in the conduction band, thereby impeding thermalization of the emissive exciton. A barrier to electron transfer must also exist in the situation in column C, because the excited electron does not thermalize to the lower-level CdS conduction band under direct excitation of the core. By analogy, the barrier to electron delocalization could also be present for tetrapods in which the absence of a shift in emission energy indicates conduction band alignment. The barrier can only exist for one of the carriers, because complete blocking at the interface would result in recombination in the CdS arm, which is spectrally distinct (fig. S8). Noting that our spectroscopy cannot distinguish between conduction and valence band blocking, we follow the earlier assignment of effective hole localization in the core of CdSe/CdS nanoparticles because of a larger offset in the valence band than in the conduction band (29). We speculate that the greater effectiveness of hole transfer across the interface as compared to electron transfer could arise from the difference in wave function symmetry of the carrier species, or result from the much larger energy-level offset.

The observation that excitation energies well above the band gap of both CdSe and CdS (that is, at 3.1 eV) do not result in complete thermalization and thus emission at the lowest energy of the system (Fig. 4, column A), which is only observed under direct core excitation, indicates that additional excitation energy does not play a role in electron transfer to the core. The CdS electron therefore cools before the CdSe/CdS interfacial exciton is formed. Our preliminary statistics on the PLE spectra (fig. S9) indicate that excitation energy-dependent emission is much more prominent in class A tetrapods. This observation is again consistent with the implied morphological disorder of the single class B tetrapod structures, because more narrowly defined (class A) energy levels should be more susceptible to band misalignment at the heterojunction.

Single-particle light-harvesting action thus reveals distinct spectroscopic behavior that is masked in the ensemble, providing insight into the uniformity of the quantum confinement and the degree of electronic delocalization across a heterojunction. Morphologies in which the quantum confinement varies within the particle (due to the formation of a CdS bulb around the CdSe core, as observed in nanorods) give rise to a lifting of the spectral signature of well-defined quantum confinement by smearing out excitonic transitions. As shown in the single-particle spectrally resolved PLE measurements, band misalignment that is more prevalent in morphologically uniform particles (class A) reveals nonthermalized interfacial excitons, which suggest the presence of an intrinsic barrier to

electron transfer between CdS and CdSe. Consequently, whereas particle uniformity and the resulting confinement may be desirable in, for example, light-emitting devices, nanoparticles with low symmetry of the confinement potential (that is, with structural variations: class B) are more suited for light-harvesting applications because of a reduced influence of interfacial barriers. The challenge posed to nanoparticle synthesis is to extend shape engineering to morphology control, so as to create samples in which, for example, all particles exhibit class B morphologies.

## References and Notes

1. X. Peng *et al.*, *Nature* **404**, 59 (2000).
2. W. U. Huynh, J. J. Dittmer, A. P. Alivisatos, *Science* **295**, 2425 (2002).
3. L. Manna, D. J. Milliron, A. Meisel, E. C. Scher, A. P. Alivisatos, *Nat. Mater.* **2**, 382 (2003).
4. T. Mokari, E. Rothenberg, I. Popov, R. Costi, U. Banin, *Science* **304**, 1787 (2004).
5. D. V. Talapin *et al.*, *Nano Lett.* **7**, 2951 (2007).
6. P. V. Kamat, *J. Phys. Chem. C* **112**, 18737 (2008).
7. A. Pandey, P. Guyot-Sionnest, *Science* **322**, 929 (2008).
8. L. Amirav, A. P. Alivisatos, *J. Phys. Chem. Lett.* **1**, 1051 (2010).
9. Y. Qu *et al.*, *Nano Lett.* **10**, 1941 (2010).
10. M. J. Walter, J. M. Lupton, *Phys. Rev. Lett.* **103**, 167401 (2009).
11. D. Steiner *et al.*, *Nano Lett.* **8**, 2954 (2008).
12. A. Sitt, F. Della Sala, G. Menagen, U. Banin, *Nano Lett.* **9**, 3470 (2009).
13. A. Pandey, P. Guyot-Sionnest, *J. Chem. Phys.* **127**, 104710 (2007).
14. J. Müller *et al.*, *Nano Lett.* **5**, 2044 (2005).
15. S. A. Empedocles, R. Neuhauser, K. Shimizu, M. G. Bawendi, *Adv. Mater.* **11**, 1243 (1999).
16. H. Htoon, P. J. Cox, V. I. Klimov, *Phys. Rev. Lett.* **93**, 187402 (2004).
17. H. Akiyama, M. Yoshita, L. N. Pfeiffer, K. W. West, A. Pinczuk, *Appl. Phys. Lett.* **82**, 379 (2003).
18. T. Ihara *et al.*, *Phys. Rev. Lett.* **99**, 126803 (2007).
19. Y. Chen *et al.*, *Appl. Phys. Lett.* **93**, 053106 (2008).
20. C. Mauser *et al.*, *Phys. Rev. B* **77**, 153303 (2008).
21. E. Yuskovitz, G. Menagen, A. Sitt, E. Lachman, U. Banin, *Nano Lett.* **10**, 3068 (2010).
22. Materials and methods are detailed in supporting material on Science Online.
23. A. Efros, M. Rosen, *Annu. Rev. Mater. Sci.* **30**, 475 (2000).
24. T. Vossmeier *et al.*, *J. Phys. Chem.* **98**, 7665 (1994).
25. M. Saba *et al.*, *Adv. Mater.* **21**, 4942 (2009).
26. J. Planellas, F. Rajadell, J. I. Climente, *J. Phys. Chem. C* **114**, 8337 (2010).
27. R. G. Neuhauser, K. T. Shimizu, W. K. Woo, S. A. Empedocles, M. G. Bawendi, *Phys. Rev. Lett.* **85**, 3301 (2000).
28. A. M. Smith, S. Nie, *Acc. Chem. Res.* **43**, 190 (2010).
29. X. Peng, M. C. Schlamp, A. V. Kadavanich, A. P. Alivisatos, *J. Am. Chem. Soc.* **119**, 7019 (1997).
30. The authors thank K. van Schooten for technical assistance and R. Polson, M. DeLong, and S. Rupich for help with SEM measurements and are indebted to NSF (grants CHE-0748473 and DMR-0213745) and the U.S. Department of Defense Office of Naval Research (grant N00014-10-1-0190). J.M.L. and D.V.T. are fellows of the David and Lucile Packard Foundation.

## Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6009/1371/DC1

Materials and Methods

SOM Text

Figs. S1 to S10

Table S1

References

21 September 2010; accepted 3 November 2010  
10.1126/science.1198070



# A Cryptic Sulfur Cycle in Oxygen-Minimum-Zone Waters off the Chilean Coast

Don E. Canfield,<sup>1\*</sup> Frank J. Stewart,<sup>2</sup> Bo Thamdrup,<sup>1</sup> Loreto De Brabandere,<sup>1</sup> Tage Dalsgaard,<sup>3</sup> Edward F. Delong,<sup>2</sup> Niels Peter Revsbech,<sup>4</sup> Osvaldo Ulloa<sup>5</sup>

Nitrogen cycling is normally thought to dominate the biogeochemistry and microbial ecology of oxygen-minimum zones in marine environments. Through a combination of molecular techniques and process rate measurements, we showed that both sulfate reduction and sulfide oxidation contribute to energy flux and elemental cycling in oxygen-free waters off the coast of northern Chile. These processes may have been overlooked because in nature, the sulfide produced by sulfate reduction immediately oxidizes back to sulfate. This cryptic sulfur cycle is linked to anammox and other nitrogen cycling processes, suggesting that it may influence biogeochemical cycling in the global ocean.

Oxygen-minimum zones (OMZs) persist in midwater depths of the global ocean, where large-scale circulation and the sinking and decomposition of surface-derived organics deplete oxygen as compared to higher-surface and deep-water oxygen concentrations (1). In some regions such as the eastern tropical Pacific, the Arabian Sea, and the Benguela Current upwelling system, water column oxygen concentrations fall below detection (2–4), prompting the development of a dynamic nitrogen cycle. In these zones, nitrate is actively reduced to nitrite (5, 6). Nitrite is further converted to  $N_2$  gas through “classic” heterotrophic denitrification (7) and the autotrophic anammox process (8, 9) or to  $NH_4^+$

through dissimilative nitrate reduction to ammonium. OMZs account for 33% or more of the loss of fixed nitrogen from the oceans (10, 11), and overall, the nitrogen cycle has been thought to dominate the geochemistry and microbial ecology of these regions.

The recent identification of uncultured *Gammaproteobacteria*, closely affiliated with sulfur-oxidizing symbionts, in OMZ waters off the Chilean coast (12) suggests that sulfur cycling may also play an important role in oxygen-free nitrate-rich OMZs. A similar microbial community with a full complement of sulfide-oxidizing and nitrate-reducing genes was found in sulfide-free but nitrate-rich portions of the sulfidic Saanich In-

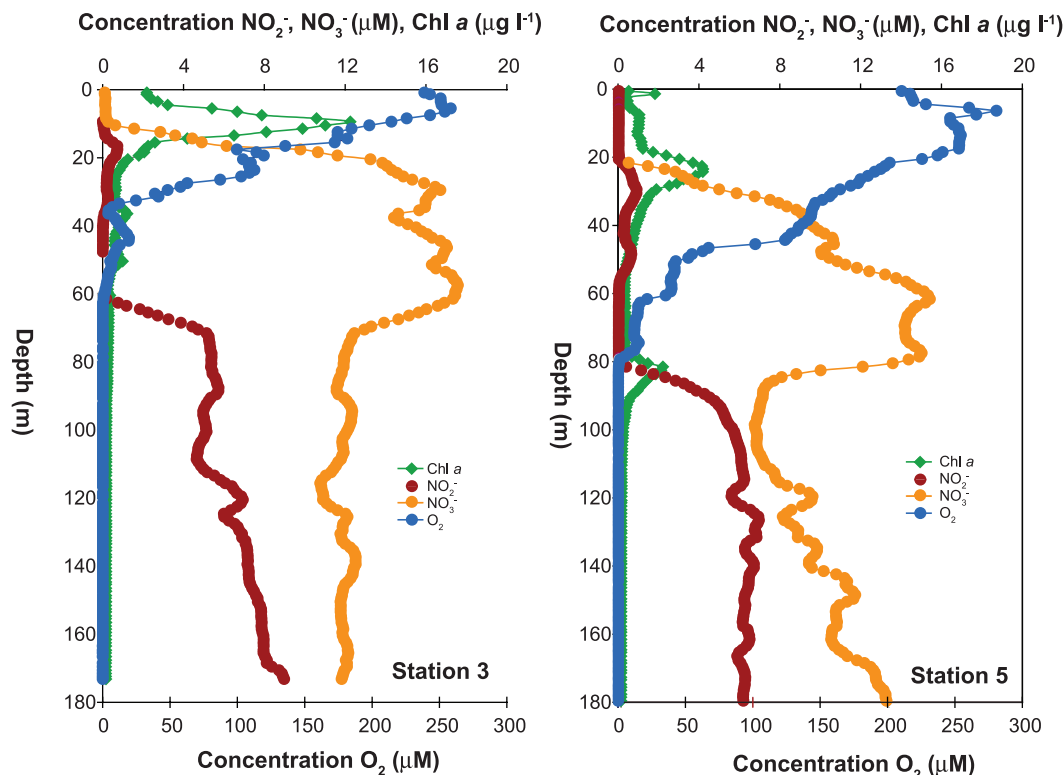
let (13), and a sulfate reducer has been isolated from OMZ waters off the coast of Peru (14). Direct evidence for large-scale active sulfur cycling in OMZs, however, is lacking. When sulfide, the product of sulfate reduction, is observed in OMZs, it originates in rare pockets of nitrate and nitrite-depleted water (15) or is released from sediments (6).

We explored the dynamics of the sulfur cycle in the upwelling waters off Iquique, on the northern Chilean coast, using a combination of geochemical and metagenomic techniques (16). In general, the OMZ is well developed in this region of Chile (17). We concentrated our efforts on station 3 (20°59.27'S, 70°20'8.18"W; water depth 1050 m, 23 km from shore), which, based on preliminary survey data, was in the most biologically active region of the OMZ in our study area. We expanded our geochemical studies to include station 5 (20°59.69'S, 70°46'5.78"W; water depth 1500 m), located some 44 km further

<sup>1</sup>Institute of Biology and Nordic Center for Earth Evolution, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark. <sup>2</sup>Department of Biological Engineering and Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, MA 02138, USA. <sup>3</sup>National Environmental Research Institute, Aarhus University, Vejlssøvej 25, Post Office Box 314, DK-8600 Silkeborg, Denmark. <sup>4</sup>Section of Microbiology, Department of Biological Sciences, Aarhus University, DK-8000 Aarhus C, Denmark. <sup>5</sup>Departamento de Oceanografía y Centro de Investigación Oceanográfica en el Pacífico Sur-Oriental, Universidad de Concepción, Casilla-160-C, Concepción, Chile.

\*To whom correspondence should be addressed. E-mail: dec@biology.sdu.dk

**Fig. 1.** Representative nutrient, oxygen, and chlorophyll *a* profiles from the OMZ off the northern Chilean coast at station 3 (left) and station 5 (right).



offshore than station 3. The water chemistry in the northern Chilean OMZ develops within an eastern boundary current, and the chemical profiles are somewhat dynamic (Fig. 1). With some variability over time, the redoxcline at station 5 was located deeper than at station 3, and although the surface concentrations of chlorophyll a were higher at station 3, we frequently observed a pronounced secondary chlorophyll a maximum at

station 5. This deep layer consists of previously unknown members of the cyanobacterial genus *Prochlorococcus* (18). We observed extremely low O<sub>2</sub> concentrations of <13 nM, starting from between 60 and 85 m depth (depending on station and time of sampling) and continuing to >180 m (fig. S1). Similar low values were found off the southern Peruvian coast in an earlier study (19), suggest-

ing that essentially anoxic waters define this region of the eastern tropical South Pacific OMZ. There is an upper nitrite maximum related to aerobic processes, but nitrite accumulated as oxygen disappeared in the anoxic core of the OMZ. Nitrate reduction was the most likely source for this nitrite. We measured with <sup>15</sup>N-enriched nitrite the rates and pathways of N<sub>2</sub> formation, and similar to what was found in an early study (8), anammox was the dominant pathway (Table 1), considerably outpacing denitrification (16). The overall rates of N<sub>2</sub> formation were similar to those in previous measurements in this region (8).

Pyrosequencing of community DNA from below the oxycline (70 to 80 m) and in the core of the anoxic zone (150 to 200 m) at station 3 suggested a substantial role for sulfur-based metabolic pathways in the Chilean OMZ (Fig. 2 and figs. S4 to S7). Gene sequences matching diverse sulfide-oxidizing and sulfate-reducing taxa (table S3) constituted 6.3 to 16.2% and 2.1 to 2.4% of all sequencing reads with matches to protein-coding genes in the National Center for Biotechnology Information–nr (NCBI-nr) database, consistent with percentages based on 16S ribosomal RNA gene-encoding reads (figs. S4 and S5). In contrast, sulfur-oxidizer and sulfate-reducer sequences represented only 0.5 and 0.3% of the total protein-coding sequences recovered in an aerobic community from another coastal site (Monterey Bay, 10 m; fig. S5). The Chilean OMZ metagenomes were particularly enriched in sequences matching the genomes of sulfur-oxidizing endosymbionts of deep-sea clams [*Candidatus* Ruthia magnifica (Rm) and *Candidatus* Vesicomysocius

Table 1. Summary of process rate averages.

|                                                                  | Station 3     | Station 5      |
|------------------------------------------------------------------|---------------|----------------|
| Process rates (nmol liter <sup>-1</sup> hour <sup>-1</sup> )     |               |                |
| Anammox                                                          | 0.43 ± 0.21†  | 0.29 ± 0.10‡   |
| Denitrification*                                                 | 0.079 ± 0.04† | 0.042 ± 0.029‡ |
| Sulfate reduction                                                | 0.51 ± 0.21§  | 0.055 ± 0.023  |
| Depth-integrated rates (mmol m <sup>-2</sup> day <sup>-1</sup> ) |               |                |
| Anammox                                                          | 1.21 ± 0.45   | 0.70 ± 0.24    |
| Denitrification                                                  | 0.22 ± 0.11   | 0.10 ± 0.069   |
| Sulfate reduction                                                | 1.00 ± 0.40¶  | 0.28 ± 0.12    |
| Carbon oxidation in OMZ #                                        | 5.50          | 5.50           |

\*Measured as nitrite reduction to N<sub>2</sub>. †From 65 to 183 m depth (n = 17). ‡From 73.5 to 173 m depth (n = 16). §From 85 to 150 m depth (n = 8). ||from 85 to 300 m depth (n = 14). ¶Assuming that sulfate reduction stops first at 200 m. #Estimated from data in (17).

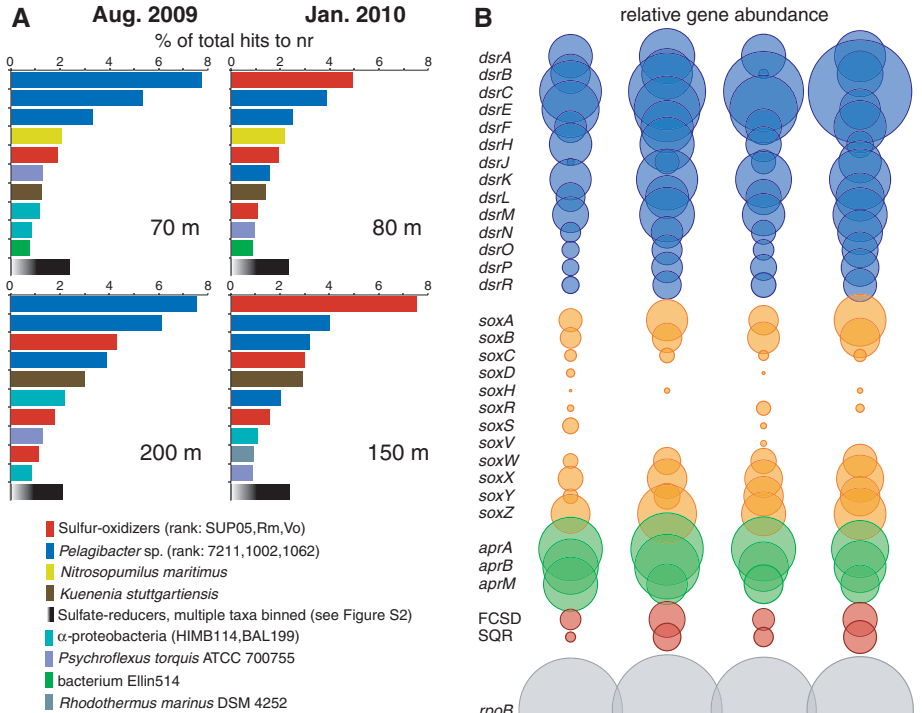


Fig. 2. Taxonomic representation of protein-coding genes and relative abundances of sulfur energy-metabolism genes in OMZ metagenomic data. (A) Most abundant taxa identified from annotations of protein-coding genes (in the NCBI-nr database) in pyrosequencing reads from genomic DNA. Reads matching multiple putative sulfate-reducer reference taxa (fig. S1 and table S2) are binned in a single category (black bar). (B) Abundances (hit counts per gene) of dissimilatory sulfur metabolism genes, shown relative to the putative single copy per organism of RNA polymerase subunit B (*rpoB*). Abundances per gene are normalized to gene length but not to copy number variation. *dsr*, dissimilatory sulfite reductase gene cluster; *sox*, sulfur oxidation gene cluster; *aprBA*, adenosine 5'-phosphosulfate (APS) reductase; *aprM*, APS reductase membrane anchor; FCSD, flavocytochrome c sulfide dehydrogenase; SQR, sulfide-quinone reductase.

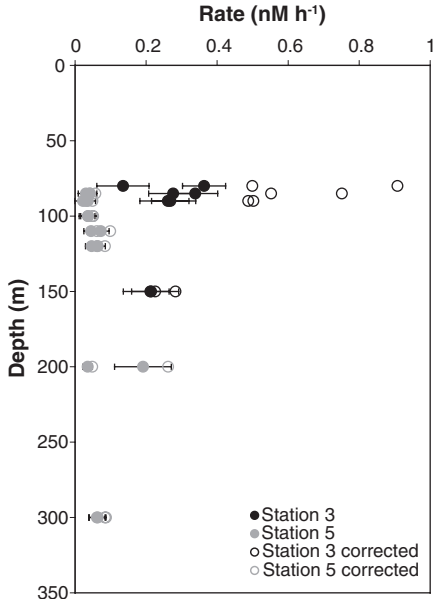


Fig. 3. Sulfide-oxidation corrected and uncorrected rates of sulfate reduction at stations 3 and 5. Standard deviations represent variability during scintillation counting (16).



okutanii (Vo) (20, 21)] and the endosymbiont-related SUP05 pelagic lineage from Saanich Inlet (13) (Fig. 2A). These taxa increased in abundance in January 2010 (austral summer) relative to samples collected from the same site in August 2009 (late winter). In the January 2010 samples, SUP05-like sequences dominated the identifiable protein-coding gene pool (up to 7.5% of all hits on NCBI-nr). The SUP05 metagenome (13) was represented at high coverage, matching 80% of all SUP05 genes (1169 of 1456) with relatively uniform abundances and an average amino acid similarity of 70% (fig. S7). The sulfate-reducing population contributed to a lower but appreciable proportion of sequencing reads and was represented by a diverse population that included *Desulfatibacillum*, *Desulfobacterium*, *Desulfococcus*, *Syntrophobacter*, and *Desulfovibrio* species (figs. S4 to S6).

The prevalence of sulfur-metabolizing taxa was paralleled by a strong representation of sulfur energy-metabolism genes. These genes occur in various combinations across diverse sulfur-utilizing taxa (22). Here, genes of the dissimilatory sulfite reductase enzyme (*dsr*), the sulfur oxidation (*sox*) gene complex mediating thiosulfate oxidation, and the adenosine 5'-phosphosulfate (APS) reductase (*apr*) were present throughout the OMZ (Fig. 2B). Several of the proteins encoded by these genes, including *dsr* and *apr* enzymes, function in both oxidative and reductive pathways (23, 24). Here, the majority of the sequences recovered in the OMZ matched known sulfide oxidizers, which is consistent with the high abundance of the SUP05 group. Putative sulfide-oxidizing and sulfate-reducing taxa constituted 62.0 and 2.2% of top hits to *aprA* sequences, respectively, with the remainder matching *aprA* genes of the alphaproteobacterial genus *Pelagibacter*, whose function in sulfide oxidation is not yet clear (25) (fig. S6). Overall, the metagenomic data suggest a prevalent summer OMZ community of both oxidative and reductive sulfur-cycling bacteria.

Although our metagenomic libraries suggest an active sulfur cycle, it is cryptic, with no obvious in situ chemical expression. To explore the geochemical importance of the sulfur cycle and possible links to nitrogen cycling, we measured rates of sulfate reduction with  $^{35}\text{SO}_4^{2-}$  (26). We subdued the immediate reoxidation of sulfide produced during sulfate reduction by adding 10 to 13  $\mu\text{M}$  unlabeled sulfide to trap any radiolabeled sulfide from sulfate reduction (16). Radiolabeled sulfate was added within 10 hours of sample collection. In some cases, our added unlabeled sulfide was substantially oxidized during the incubations (16), implying that radiolabeled sulfide must also have been oxidized and lost as a result. After estimating the loss of radiolabeled sulfide due to sulfide oxidation, we corrected the rates to obtain estimates of the gross sulfate reduction rates (16) (Fig. 3). Our findings contrast with

the current consensus that sulfate reduction in OMZs will be active only when other more thermodynamically favorable electron acceptors, such as nitrate and nitrite, are fully utilized (27). Although not the most favorable, our calculations show that sulfate reduction is still a thermodynamically favorable process in these OMZ waters (16). Previous observations of pure cultures of sulfate-reducing bacteria that actively reduce sulfate in the presence of nitrate (28, 29) also support our observations of active sulfate reduction.

Rates of sulfate reduction were much higher at station 3 than at station 5. Indeed, corrected rates at station 3 match and even exceed rates of denitrification and anammox (Table 1), implying that sulfate reduction is an important pathway of organic carbon mineralization at this site. Depth-integrated corrected rates of sulfate reduction at station 3 are equivalent to about 2 mmol of C oxidized  $\text{m}^{-2} \text{day}^{-1}$ , assuming 2 mol of organic carbon oxidized per mole of sulfate reduced. Sediment trap studies at coastal and offshore stations about 200 km south of our study site (17) reveal about 5.50 mmol  $\text{m}^{-2} \text{day}^{-1}$  of carbon mineralization within the OMZ waters from between 65 and 300 m depth. If these rates apply to station 3, then sulfate reduction would account for about 33% of the total organic carbon mineralization in the OMZ waters.

Sulfate reduction may also contribute to the ammonium requirements of other indigenous bacteria participating in the anammox process. Indeed, the source of ammonium for anammox has proven elusive because insufficient ammonium is liberated during organic matter decomposition by denitrification to drive measured anammox rates in many OMZ waters (8, 9). In a partial resolution to this dilemma, the dissimilatory reduction of nitrate to ammonium and the heterotrophic reduction of nitrate to nitrite have been identified as significant ammonium sources in OMZ waters off the Peruvian coast (9) (the latter due to the ammonium liberated during mineralization of organic matter). But even these extra sources do not account for all of the ammonium demand. From our sulfate reduction rates at station 3, sulfate reduction produces a total of about 0.30 mmol  $\text{m}^{-2} \text{day}^{-1}$ , assuming a 6.6/1 ratio between carbon oxidation and ammonium liberation (30). This would contribute 22% of the ammonium needs for anammox at station 3 (Table 1). At station 5, sulfate reduction would contribute only about 8% of the ammonium needs for anammox, underlining the complexity of the nitrogen cycle and the variability of ammonium sources for anammox (9).

We also explored the dynamics of sulfide oxidation in these waters and the relationship between sulfide oxidation and the nitrogen cycle (16). In parallel with our sulfate reduction rate determinations, we incubated OMZ water from two depths at both stations 3 and 5 with and without added sulfide. Sulfide oxidation was strongly coupled to nitrate reduction to nitrite, and at sta-

tion 5, nitrate reduction to nitrous oxide was also enhanced with sulfide addition (fig. S8). At station 3,  $\text{N}_2$  production from both nitrite and nitrate (at 75 m depth) increased, and in general, rates of sulfide oxidation and subsequent rates of nitrogen turnover were much higher at station 3 than at station 5. This is consistent with the higher rates of sulfate reduction at station 3 and a more active sulfur cycle.

Admittedly, our added levels of sulfide and subsequent rates of sulfide oxidation exceed in situ levels. Nevertheless, our results demonstrate the inherent capacity for active in situ coupling between the sulfur and nitrogen cycles in OMZ zones of the marine water column. This cycling is analogous to that observed at the sulfide/nitrate interface in other strongly redox stratified marine systems (13, 31, 32) and demonstrates that nitrite,  $\text{N}_2$ , and  $\text{N}_2\text{O}$  may all be products of this coupling. We speculate that other nitrate-rich oxygen-free OMZs may also house actively coupled sulfur and nitrogen cycles.

## References and Notes

1. K. Wyrski, *Deep-Sea Res.* **9**, 11 (1962).
2. J. D. Cline, F. A. Richards, *Limnol. Oceanogr.* **17**, 885 (1972).
3. D. B. Olson, G. L. Hitchcock, R. A. Fine, B. A. Warren, *Deep Sea Res. Part II Top. Stud. Oceanogr.* **40**, 673 (1993).
4. S. E. Calvert, N. B. Price, *Deep-Sea Res.* **18**, 505 (1971).
5. J. M. Morrison et al., *Deep Sea Res. Part II Top. Stud. Oceanogr.* **46**, 1903 (1999).
6. V. Bruchert et al., *Geochim. Cosmochim. Acta* **67**, 4505 (2003).
7. S. E. Bulow, J. J. Rich, H. S. Naik, A. K. Pratihary, B. B. Ward, *Deep Sea Res. Part I Oceanogr. Res. Pap.* **57**, 384 (2010).
8. B. Thamdrup et al., *Limnol. Oceanogr.* **51**, 2145 (2006).
9. P. Lam et al., *Proc. Natl. Acad. Sci. U.S.A.* **106**, 4752 (2009).
10. L. A. Codispoti et al., *Sci. Mar.* **65**, 85 (2001).
11. J. N. Galloway et al., *Biogeochemistry* **70**, 153 (2004).
12. H. Stevens, O. Ulloa, *Environ. Microbiol.* **10**, 1244 (2008).
13. D. A. Walsh et al., *Science* **326**, 578 (2009).
14. K. W. Finster, K. U. Kjeldsen, *Antonie van Leeuwenhoek* **97**, 221 (2010).
15. R. C. Dugdale, J. J. Goering, R. T. Barber, R. L. Smith, T. T. Packard, *Deep-Sea Res.* **24**, 601 (1977).
16. See supporting material on Science Online.
17. R. Escubano et al., *Deep Sea Res. Part II Top. Stud. Oceanogr.* **51**, 2389 (2004).
18. P. Lavín, B. González, J. F. Santibáñez, D. J. Scanlan, O. Ulloa, *Environ. Microbiol. Rep.* 10.1111/j.1758-2229.2010.00167.x (2010).
19. N. P. Revsbech et al., *Limnol. Oceanogr. Methods* **7**, 371 (2009).
20. I. L. G. Newton et al., *Science* **315**, 998 (2007).
21. H. Kuwahara et al., *Curr. Biol.* **17**, 881 (2007).
22. C. Dahl, C. G. Friedrich, Eds., *Microbial Sulfur Metabolism* (Springer-Verlag, Heidelberg, 2008).
23. B. Meyer, J. Kuever, *Microbiology* **153**, 3478 (2007).
24. B. Meyer, J. Kuever, *Microbiology* **153**, 2026 (2007).
25. B. Meyer, J. Kuever, *Appl. Environ. Microbiol.* **73**, 7664 (2007).
26. D. B. Albert, C. Taylor, C. S. Martens, *Deep-Sea Res.* **42**, 1239 (1995).
27. P. N. Froelich et al., *Geochim. Cosmochim. Acta* **43**, 1075 (1979).

28. T. Dalsgaard, F. Bak, *Appl. Environ. Microbiol.* **60**, 291 (1994).
29. R. G. L. McCready, W. D. Gould, F. D. Cook, *Arch. Microbiol.* **135**, 182 (1983).
30. A. C. Redfield, B. H. Ketchum, F. A. Richards, in *The Sea*, N. M. Hill, Ed. (Academic Press, London, 1963), vol. 2, pp. 26–77.
31. M. M. Jensen, J. Petersen, T. Dalsgaard, B. Thamdrup, *Mar. Chem.* **113**, 102 (2009).
32. G. Lavik *et al.*, *Nature* **457**, 581 (2009).
33. We thank the captain and crew of the *Agor Vidal Gormaz* from the Chilean Navy for their kind support, and the Agouron Institute, the Danish National Research Foundation, the Gordon and Betty Moore Foundation, and the Chilean Fondap Program for financial support. Additional thanks to G. Alarcón, G. Friederich, and J. Jennings for operational and experimental support. The genome sequence data are accessible on NCBI's Sequence Read Archive via accession number SRA025088.

## Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1196889/DC1  
Materials and Methods  
Figs. S1 to S8  
Tables S1 to S4  
References

24 August 2010; accepted 28 October 2010  
Published online 11 November 2010;  
10.1126/science.1196889

# Dynamical Response of the Tropical Pacific Ocean to Solar Forcing During the Early Holocene

Thomas M. Marchitto,<sup>1\*</sup> Raimund Muscheler,<sup>2</sup> Joseph D. Ortiz,<sup>3</sup>  
Jose D. Carriquiry,<sup>4</sup> Alexander van Geen<sup>5</sup>

We present a high-resolution magnesium/calcium proxy record of Holocene sea surface temperature (SST) from off the west coast of Baja California Sur, Mexico, a region where interannual SST variability is dominated today by the influence of the El Niño–Southern Oscillation (ENSO). Temperatures were lowest during the early to middle Holocene, consistent with documented eastern equatorial Pacific cooling and numerical model simulations of orbital forcing into a La Niña-like state at that time. The early Holocene SSTs were also characterized by millennial-scale fluctuations that correlate with cosmogenic nuclide proxies of solar variability, with inferred solar minima corresponding to El Niño-like (warm) conditions, in apparent agreement with the theoretical “ocean dynamical thermostat” response of ENSO to exogenous radiative forcing.

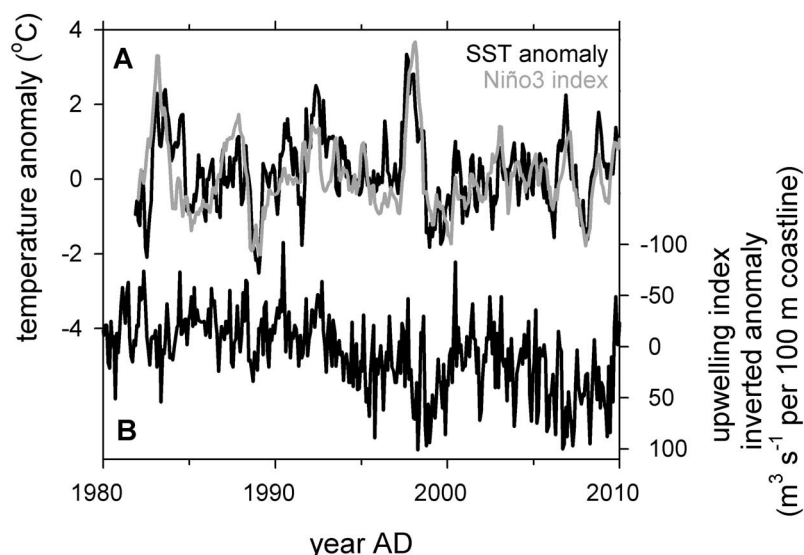
The influence of solar variability on Earth's climate over centennial to millennial time scales is the subject of considerable debate. The change in total solar irradiance over recent 11-year sunspot cycles amounts to <0.1%, but greater changes at ultraviolet wavelengths (*1*) may have substantial impacts on stratospheric ozone concentrations, thereby altering both stratospheric and tropospheric circulation patterns (*2*). Estimates of the secular increase in total irradiance since the late 17th century Maunder sunspot minimum range from ~0.05 to 0.5% (*1*). Values in the middle of this range are sufficient to force the intermediate-complexity Zebiak-Cane model of El Niño–Southern Oscillation (ENSO) dynamics into a more El Niño-like state during the Little Ice Age (A.D. ~1400 to 1850) (*3*), a response dubbed the “ocean dynamical thermostat” because negative (or positive) radiative forcing results in dynamical ocean warming (or cooling, respectively) of the eastern tropical Pacific (ETP) (*4*). This model prediction is supported by paleoclimatic proxy reconstructions over the past millennium (*3, 5, 6*). In contrast, fully coupled general circulation models (GCMs) lack a robust thermostat response because of an oppo-

sing tendency for the atmospheric circulation itself to strengthen under reduced radiative forcing (*7*).

ENSO is a leading source of interannual climate variability over large regions of the globe, so it is crucial to gain an improved understanding of its past responses to external forcing at various time scales. Tropical fossil corals provide the most reliable means for reconstructing ENSO conditions from the past (*5*), but the record is currently too

fragmented to test for any relation to persistent solar forcing before the past millennium. Few sea surface temperature (SST) reconstructions from well-placed tropical Pacific sediment cores have sufficient temporal resolution to address this question.

Sediment core composite MV99-GC41/PC14 was raised from a water depth of 540 m on the floor of Soledad Basin, which is located off the west coast of Baja California Sur, Mexico (25.2°N, 112.7°W), and has an effective sill depth of 290 m (*8*). Although this site is just outside of the tropical band, modern conditions here are strongly teleconnected to ENSO. The modern annual cycle of SST has an amplitude of ~8°C on average, with minimum temperatures during spring, the season of strongest coastal upwelling (*9, 10*). Yet interannual variability in SST is much more strongly dependent on ENSO than on local upwelling winds. Over the 30-year period covered by satellite observations, the Niño 3 index explains 37% of the monthly SST anomaly near Soledad Basin (correlation coefficient  $r = 0.61$  maximum correlation with a 2-month lag), whereas the local upwelling index explains only 2% ( $r = -0.16$  with zero lag) (Fig. 1). ENSO is crucial for SST because the regional thermocline deepens during El Niño years, so that even with vigorous local upwelling the ascending waters are warmer than during La Niña or neutral years (*11*). Recent spring SST minima have ranged from 17°C during strong La Niñas to 20°C during



**Fig. 1.** (A) Monthly SST anomaly for the 1° grid cell situated over Soledad Basin (black) (*9*), compared to the monthly Niño 3 SST index on the same vertical scale but lagged by 2 months (gray) (*9*) and (B) the local (24°N, 113°W) monthly upwelling index (offshore Ekman transport computed from wind stress) anomaly (*10*), shown inverted.

<sup>1</sup>Department of Geological Sciences and Institute of Arctic and Alpine Research, University of Colorado, Boulder, CO 80309, USA.

<sup>2</sup>Department of Earth and Ecosystem Sciences, Lund University, S-223 62 Lund, Sweden. <sup>3</sup>Department of Geology, Kent State University, Kent, OH 44242, USA. <sup>4</sup>Department of Environmental Geosciences, Universidad Autónoma de Baja California, Ensenada, Baja California 22830, Mexico. <sup>5</sup>Lamont-Doherty Earth Observatory, Columbia University, Palisades, NY 10964, USA.

\*To whom correspondence should be addressed. E-mail: tom.marchitto@colorado.edu



El Niños. Surface warming off the coast of Baja California Sur can be further enhanced under El Niño conditions by the northeastward expansion of subtropical surface waters that effectively block the admixture of southward-flowing subarctic (California Current) waters (12).

An age model based on 22 calibrated accelerator mass spectrometry radiocarbon dates (fig. S1) reveals that the composite core spans the past 13.9 thousand years (ky) with an average sedimentation rate of  $>1 \text{ m ky}^{-1}$ . During the Holocene, the sediments are laminated, indicating negligible bioturbation under low- $\text{O}_2$  conditions on the sea floor (8). Preservation of planktonic foraminifera is excellent throughout the core, with glassy tests and spines commonly present. We measured the SST proxy Mg/Ca in the planktonic foraminifer *Globigerina bulloides* (13), which lives at the sea surface primarily during the spring peak upwelling season along this margin (14). Samples were nominally spaced at 5-cm intervals and contained 30 to 60 foraminifera each, so each measurement theoretically averages 30 to 60 month-long (foraminiferal lifespan) upwelling-season snapshots spread over roughly a decade (1-cm sample width), with  $\sim 50$ -year spacing between samples. Although not capable of resolving the typical ENSO periodicities of 2 to 7 years, this sampling is sufficient to detect any multicentennial/millennial-scale changes in spring SSTs.

Our Mg/Ca-based SST reconstruction indicates that early to middle Holocene [ $\sim 4$  to 10 thousand years ago (ka)] spring temperatures were  $\sim 1^\circ\text{C}$  cooler, on average, than during the rest of the past 14 ky (Fig. 2). By analogy with modern ETP dynamics, we suggest that the cooling is best explained by a shallower thermocline and a reduced influence of subtropical surface waters. This scenario is consistent with previous suggestions of a more La Niña-like state during the early to middle Holocene. Mg/Ca reconstructions from the equatorial Pacific indicate an enhanced zonal SST gradient at this time, with a colder eastern cold tongue and warmer western warm pool (Fig. 2) (15, 16). At Baja California Sur, the cooling may have been amplified by a strengthened California Current (17). In contrast, alkenone-based SST reconstructions from both Baja California Sur (18) and the cold tongue (19) do not exhibit a mid-Holocene cooling. This disparity might be due to a summer/fall habitat for coccolithophores, resulting in an overprinting of La Niña-like cooling by orbitally forced seasonal radiative heating (20).

Numerical models of varying complexity have simulated a La Niña-like cooling of the ETP during the early to middle Holocene in response to enhanced boreal summer/fall insolation. Easterly winds strengthen because of zonally asymmetric heating of the tropical ocean and atmosphere

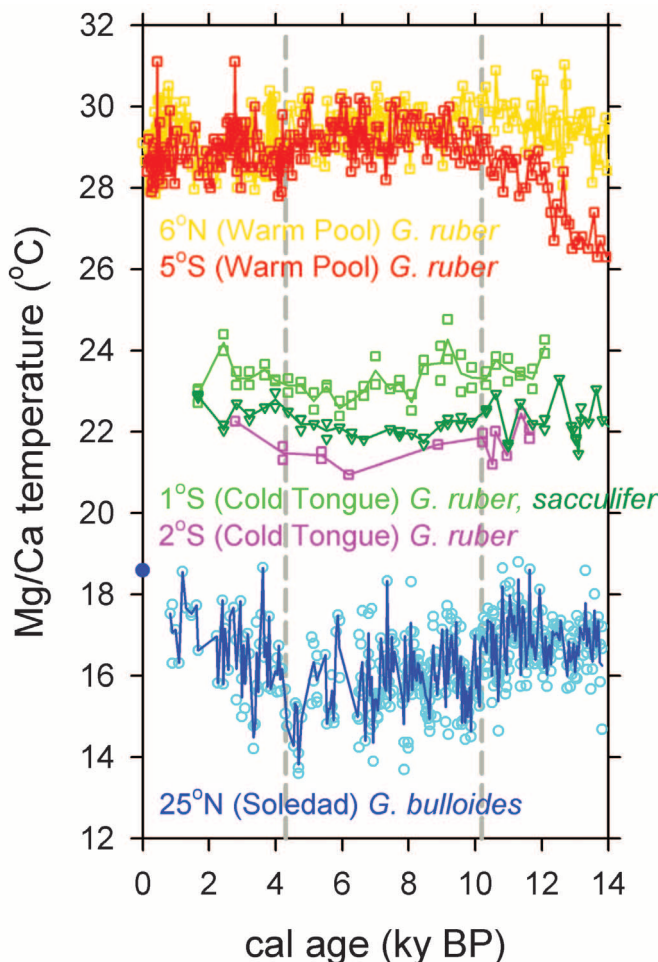
(21, 22), increased atmospheric baroclinicity (23), and/or intensification of the Asian summer monsoon (24). Although local Baja California Sur upwelling-favorable winds may also respond positively to orbital forcing, the maximum in spring insolation occurred much earlier ( $\sim 15$  ka) than our observed cooling. In support of more distant teleconnections, the shift toward warmer upwelling conditions just before 4 ka is close to the timing of widespread evidence for an abrupt and permanent weakening of the Asian summer monsoons (25), which may have helped the Pacific relax into a more El Niño-like state (24).

In addition to orbital-scale changes, the Soledad Basin Mg/Ca record displays strong variance at millennial time scales, as seen in the five-depth (nominally 200 to 300 years) running mean of the Mg/Ca data (Fig. 3). Before the data gap at  $\sim 5.9$  to 6.5 ka, sample density is high enough (48 measurements per thousand years), and the relative noise low enough, to give us confidence that the smoothed record captures a meaningful millennial-scale climate history. We observe five cold intervals between  $\sim 7$  and 11 ka, with roughly 1-ky spacing. In light of model- and proxy-based results supporting a solar influence on ENSO over the past millennium (3, 6), we compare the smoothed record to cosmogenic nuclide proxies for solar activity.

For the period before the beginning of sunspot observations in A.D. 1610, reconstructions of solar variability are based on the cosmogenic nuclides  $^{14}\text{C}$  (recorded in tree rings) (26) and  $^{10}\text{Be}$  (preserved in polar ice cores) (27, 28). An active Sun generates a higher total irradiance and a stronger interplanetary magnetic field that helps to shield Earth from the galactic cosmic rays that produce  $^{14}\text{C}$  and  $^{10}\text{Be}$  in the atmosphere. However, the relation between solar irradiance and cosmic-ray shielding is not well understood over long time scales. In addition, atmospheric levels of  $^{14}\text{C}$  may be affected by changes in Earth's carbon cycle,  $^{10}\text{Be}$  fluxes to ice sheets may be influenced by local climate, and the production rates of both nuclides are modulated by long-term variations in Earth's magnetic field. Nevertheless, the shared variance of high-pass-filtered (to correct for presumed slow variations in the geomagnetic field)  $^{14}\text{C}$  and  $^{10}\text{Be}$  records can be taken as an indication of fluctuations in solar activity over the Holocene.

Each of the early Holocene millennial-scale coolings at Soledad Basin corresponds to an inferred millennial-scale increase in solar activity (decreased cosmogenic nuclides) (Fig. 3). Cross-wavelet analysis of the unsmoothed data indicates significant common power (in phase) between Mg/Ca and the nuclides in the  $\sim 800$ - to 1000-year band (fig. S2). After performing a  $\sim 250$ -year smoothing and  $\frac{1}{1800} \text{ year}^{-1}$  high-pass filtering of each record, Mg/Ca (before the  $\sim 5.9$ -6.5 ka data gap) correlates significantly with  $^{14}\text{C}$  production ( $r = 0.49$ ,  $p = 0.02$ , with 50-year lag on Mg/Ca) and reasonably well with  $^{10}\text{Be}$  flux ( $r = 0.41$ ,  $p = 0.07$ , with 100-year lag on Mg/Ca) (Fig. 4) (13). These correlations are based on completely independent age models. Given the strong link between this region and ENSO variability today, we suggest that this correspondence

**Fig. 2.** SST reconstructions based on Mg/Ca in surface-dwelling planktonic foraminifera from the western equatorial Pacific warm pool (15) (gold and red), eastern equatorial Pacific cold tongue (16) (green and pink), and Soledad Basin (this study) (blue). Symbols denote individual measurements, and lines trace the mean at each depth. Along the equator, *G. ruber* and *G. sacculifer* are believed to represent mean annual conditions, whereas at Soledad Basin, *G. bulloides* reflects spring upwelling. The solid blue circle on the y axis denotes the modern average SST during the coldest month of the year (spring peak upwelling) at Soledad Basin (9). Vertical gray dashed lines bracket the early to middle Holocene interval of increased zonal SST gradient. BP, before the present.



provides support for the idea that the ocean dynamical thermostat (4) acts effectively at centennial-millennial time scales (3). Indeed, these early Holocene oscillations between warm El Niño-like and cool La Niña-like conditions were recently predicted by solar-forcing experiments using the Zebiak-Cane model (29). Although it is possible that local upwelling-favorable winds responded directly to positive solar forcing and amplified the cool SST signal, we argue, on the basis of modern observations (Fig. 1), that the impact would have been minor without a concomitant La Niña-like shoaling of the regional thermocline. Between ~2.2 and 5.9 ka, the poor

correlation between Mg/Ca and the solar proxies may be due to the lower sample density (less than half that of the earlier interval) and/or the reduced amplitude of inferred solar variability, in line with the model prediction (29).

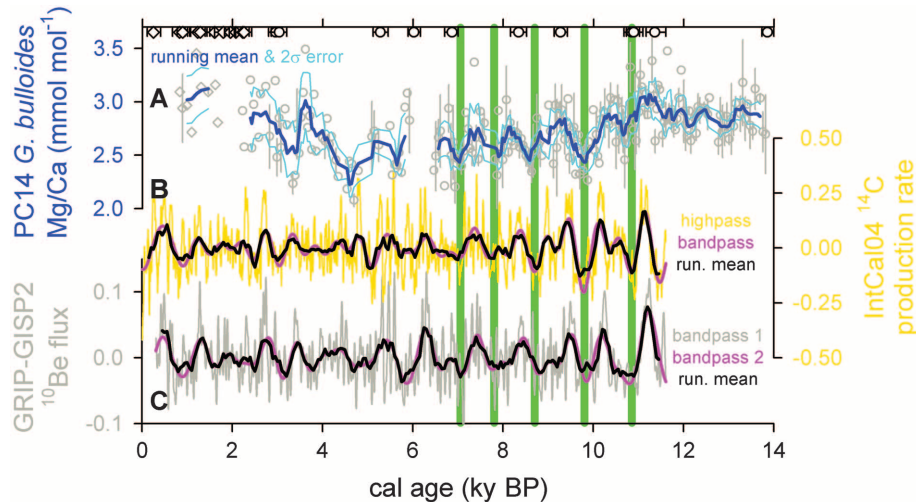
The observed sensitivity of the tropical Pacific to modest radiative forcing may have been achieved through positive feedback with other regions that also responded to solar variability. La Niña has historically been associated with stronger summer monsoons over Asia, as both are linked to strong easterlies over the tropical Pacific (30). Oxygen isotopes from speleothems in southern China (31) and Oman (32) indicate monsoon

strengthening during early Holocene solar maxima, suggesting that an Asian teleconnection may have helped push the Pacific into a La Niña-like state during these intervals, or vice versa. Although the period of overlap is relatively short, the smoothed speleothem records bear strong resemblance to the Soledad Basin SST history (China:  $r = 0.74$ ,  $p = 0.01$ ; Oman:  $r = 0.76$ ,  $p = 0.003$ ) (Fig. 4). It is interesting to note that during the interval of greatest mismatch between Soledad Basin and the solar proxies, the cave records agree with our SST history: At 8.2 ka, the ETP was in an El Niño-like state and the monsoons were weak, despite the inferred secular increase in solar activity. This apparent anomaly may be attributed to the well-known “8.2-ka event,” during which a large Laurentide meltwater discharge is believed to have cooled the North Atlantic and Eurasia, thereby weakening the Asian summer monsoons (33), which possibly fostered El Niño-like conditions in the ETP.

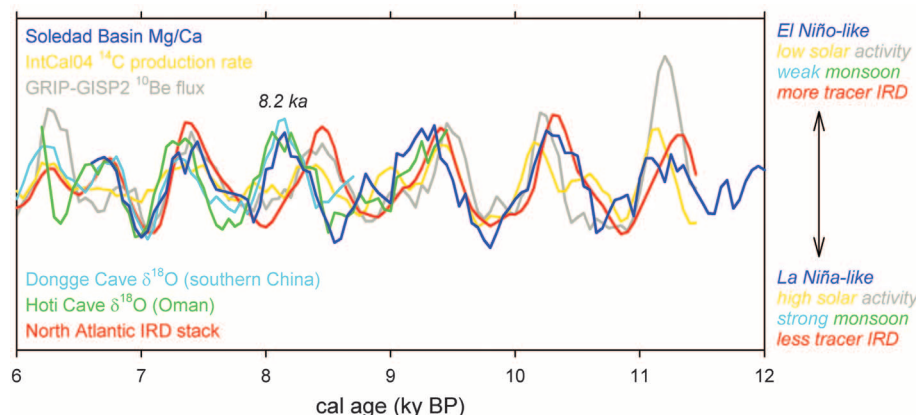
Additionally, Bond *et al.* (34) showed that there was increased ice-rafter debris (IRD) delivery from the Labrador and Nordic Seas into the North Atlantic during inferred Holocene solar minima. Their stacked IRD record correlates with Soledad Basin SSTs even more strongly than do the solar proxies ( $r = 0.70$ ,  $p < 0.001$ , with 100-year lag on Mg/Ca) (Fig. 4). A cold North Atlantic during solar minima may have reinforced ETP warming through either the Asian monsoon linkage (24) or a southward shift of the intertropical convergence zone (16). Closure of this hypothetical positive feedback loop has been suggested to occur through an El Niño-forced shift in the prevailing winds that deliver drift ice from the Nordic Seas into the North Atlantic (29).

Persistent, decadal-scale droughts over the western United States have been linked to La Niña-like SST patterns in the ETP during the instrumental period (35). Tree-ring reconstructions extend this relationship back to the Medieval Warm Period (MWP, A.D. ~900 to 1300), which was seemingly characterized by positive solar forcing, inactive tropical volcanism, La Niña-like conditions, and multidecadal “megadroughts” (3, 5, 6, 35). The first high-resolution, continuous Holocene speleothem proxy precipitation record from the southwestern United States documents a robust connection between inferred solar-activity maxima and dry conditions, which may be explained by solar forcing of La Niña-like states (36). Taken together with our SST record, these observations are consistent with solar-induced dynamical cooling of the ETP and provide predictions for millennial-scale fluctuations in the hydrologic balance over the western United States during the early Holocene.

GCMs fail to reproduce the La Niña-like nature of the MWP because the ocean thermostat mechanism is either absent or dampened by atmospheric effects in such models (6, 7). If our observations are supported by future SST reconstructions from the equatorial Pacific, then it is possible that the sensitivity of the climate system to solar forcing is underestimated by current GCMs. The nature of the climate response appears to be one of



**Fig. 3.** Soledad Basin Mg/Ca record compared to solar proxies  $^{14}\text{C}$  and  $^{10}\text{Be}$  (13). (A) *G. bulloides* Mg/Ca mean and standard deviation at each depth (gray) with five-depth running mean (blue) and associated  $2\sigma$  uncertainty estimate (light blue). Open black symbols at the top of the figure denote calibrated  $^{14}\text{C}$  ages with  $1\sigma$  errors. Diamonds are from GC41, and circles are from PC14. (B) Holocene tree-ring-derived  $\Delta^{14}\text{C}$  (26) converted to  $^{14}\text{C}$  production rate, with high values corresponding to low inferred solar activity. Data were high-pass filtered at  $\frac{1}{1800}$  year $^{-1}$  to remove secular changes that are probably related to Earth's magnetic field (gold), band-pass filtered at  $\frac{1}{1800}$  to  $\frac{1}{500}$  year $^{-1}$  as in (34) and smoothed with a 250-year running mean before  $\frac{1}{1800}$  year $^{-1}$  high-pass filtering (black). (C) Holocene ice core  $^{10}\text{Be}$  flux (27, 28) filtered as in (B), except that the gray curve is a  $\frac{1}{1800}$  to  $\frac{1}{50}$  year $^{-1}$  band pass that additionally eliminates subdecadal-scale noise. Green vertical lines indicate Soledad Basin cold intervals that correspond to times of increased solar activity.



**Fig. 4.** Likely teleconnected climatic and solar proxy records spanning the early Holocene, each smoothed at ~250 years and high-pass filtered at  $\frac{1}{1800}$  year $^{-1}$  (13). Records are Soledad Basin *G. bulloides* Mg/Ca (this study) (blue), tree-ring-derived (26)  $^{14}\text{C}$  production rate (gold), ice core  $^{10}\text{Be}$  flux (27, 28) (gray), Dongge Cave (southern China) stalagmite  $\delta^{18}\text{O}$  (31) (light blue), Hoti Cave (Oman) stalagmite  $\delta^{18}\text{O}$  (32) (green), and North Atlantic stack of IRD petrologic tracers (34) (red). All records are on their independent and untuned age models.



shifting atmosphere-ocean circulation patterns, with the tendency for global radiative surface warming being countered by the ocean dynamical thermostat.

## References and Notes

- J. L. Lean, *Geophys. Res. Lett.* **27**, 2425 (2000).
- G. A. Meehl, J. M. Arblaster, K. Matthes, F. Sassi, H. van Loon, *Science* **325**, 1114 (2009).
- M. E. Mann, M. A. Cane, S. E. Zebiak, A. Clement, *J. Clim.* **18**, 447 (2005).
- A. C. Clement, R. Seager, M. A. Cane, S. E. Zebiak, *J. Clim.* **9**, 2190 (1996).
- K. M. Cobb, C. D. Charles, H. Cheng, R. L. Edwards, *Nature* **424**, 271 (2003).
- M. E. Mann *et al.*, *Science* **326**, 1256 (2009).
- G. A. Vecchi, A. Clement, B. J. Soden, *Eos* **89**, 81 (2008).
- A. van Geen *et al.*, *Paleoceanography* **18**, 1098 (2003).
- R. W. Reynolds, N. A. Rayner, T. M. Smith, D. C. Stokes, W. Q. Wang, *J. Clim.* **15**, 1609 (2002).
- F. B. Schwing, M. O'Farrell, J. M. Steger, K. Baltz, "Coastal Upwelling Indices West Coast of North America 1946-96," *NOAA Tech. Memo. NOAA-TM-NMFS-SWSFC-231* [National Oceanic and Atmospheric Administration (NOAA), Washington, DC, 1996].
- F. B. Schwing, T. Murphree, L. deWitt, P. M. Green, *Prog. Oceanogr.* **54**, 459 (2002).
- R. Durazo, T. R. Baumgartner, *Prog. Oceanogr.* **54**, 7 (2002).
- Methods are available as supporting material on *Science* Online.
- L. R. Sautter, R. C. Thunell, *Paleoceanography* **6**, 307 (1991).
- L. Stott *et al.*, *Nature* **431**, 56 (2004).
- A. Koutavas, P. B. deMenocal, G. C. Olive, J. Lynch-Stieglitz, *Geology* **34**, 993 (2006).
- J. A. Barron, D. Bukry, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **248**, 313 (2007).
- T. D. Herbert *et al.*, *Science* **293**, 71 (2001); 10.1126/science.1059209.
- A. Koutavas, J. P. Sachs, *Paleoceanography* **23**, PA4205 (2008).
- G. Leduc, R. Schneider, J. H. Kim, G. Lohmann, *Quat. Sci. Rev.* **29**, 989 (2010).
- A. C. Clement, R. Seager, M. A. Cane, *Paleoceanography* **15**, 731 (2000).
- B. L. Otto-Bliesner, E. C. Brady, S.-I. Shin, Z. Liu, C. Shields, *Geophys. Res. Lett.* **30**, 2198 (2003).
- A. B. G. Bush, *Geophys. Res. Lett.* **26**, 99 (1999).
- Z. Liu, J. Kutzbach, L. Wu, *Geophys. Res. Lett.* **27**, 2265 (2000).
- C. Morrill, J. T. Overpeck, J. E. Cole, *Holocene* **13**, 465 (2003).
- P. J. Reimer *et al.*, *Radiocarbon* **46**, 1029 (2004).
- R. C. Finkel, K. Nishiizumi, *J. Geophys. Res. Oceans* **102**, 26699 (1997).
- M. Vonmoos, J. Beer, R. Muscheler, *J. Geophys. Res. Space Phys.* **111**, A10105 (2006).
- J. Emile-Geay, M. Cane, R. Seager, A. Kaplan, P. Almasi, *Paleoceanography* **22**, PA3210 (2007).
- P. J. Webster *et al.*, *J. Geophys. Res.* **103**, 14451 (1998).
- Y. J. Wang *et al.*, *Science* **308**, 854 (2005).
- U. Neff *et al.*, *Nature* **411**, 290 (2001).
- R. B. Alley *et al.*, *Geology* **25**, 483 (1997).
- G. Bond *et al.*, *Science* **294**, 2130 (2001); 10.1126/science.1065680.
- E. R. Cook, R. Seager, M. A. Cane, D. W. Stahle, *Earth Sci. Rev.* **81**, 93 (2007).
- Y. Asmerom, V. Polyak, S. Burns, J. Rasmussen, *Geology* **35**, 1 (2007).
- We thank P. Cappa, D. Lopez, D. Weller, and C. Wolak for laboratory assistance. This manuscript was improved by comments from B. Fox-Kemper and S. Lehman. This work was supported by collaborative NSF grants OCE-0214221 and OCE-0214646. R.M. is supported by the Royal Swedish Academy of Sciences through a grant financed by the Knut and Alice Wallenberg Foundation. Data are available at the NOAA National Climatic Data Center for Paleoclimatology.

## Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6009/1378/DC1  
Methods  
SOM Text  
Figs. S1 to S3  
Table S1  
References

9 July 2010; accepted 29 October 2010  
10.1126/science.1194887

# Plasticity of Animal Genome Architecture Unmasked by Rapid Evolution of a Pelagic Tunicate

France Denoeud,<sup>1,2,3</sup> Simon Henriët,<sup>4\*</sup> Sutada Mungpakdee,<sup>4\*</sup> Jean-Marc Aury,<sup>1,2,3\*</sup> Corinne Da Silva,<sup>1,2,3\*</sup> Henner Brinkmann,<sup>5</sup> Jana Mikhaleva,<sup>4</sup> Lisbeth Charlotte Olsen,<sup>4</sup> Claire Jubin,<sup>1,2,3</sup> Cristian Cañestro,<sup>6,24</sup> Jean-Marie Bouquet,<sup>4</sup> Gemma Danks,<sup>4,7</sup> Julie Poulain,<sup>1,2,3</sup> Coen Campsteijn,<sup>4</sup> Marcin Adamski,<sup>4</sup> Ismael Cross,<sup>8</sup> Fekadu Yadetie,<sup>4</sup> Matthieu Muffato,<sup>9</sup> Alexandra Louis,<sup>9</sup> Stephen Butcher,<sup>10</sup> Georgia Tsagkogeorga,<sup>11</sup> Anke Konrad,<sup>22</sup> Sarabdeep Singh,<sup>12</sup> Marit Flo Jensen,<sup>4</sup> Evelyn Huynh Cong,<sup>4</sup> Helen Eikeseth-Otteraa,<sup>4</sup> Benjamin Noel,<sup>1,2,3</sup> Véronique Anhouard,<sup>1,2,3</sup> Betina M. Porcel,<sup>1,2,3</sup> Rym Kachouri-Lafond,<sup>13</sup> Atsuo Nishino,<sup>14</sup> Matteo Ugolini,<sup>4</sup> Pascal Chourrout,<sup>15</sup> Hiroki Nishida,<sup>14</sup> Rein Aasland,<sup>16</sup> Snehalata Huzurbazar,<sup>12</sup> Eric Westhof,<sup>13</sup> Frédéric Delsuc,<sup>11</sup> Hans Lehrach,<sup>17</sup> Richard Reinhardt,<sup>17</sup> Jean Weissenbach,<sup>1,2,3</sup> Scott W. Roy,<sup>18</sup> François Artiguenave,<sup>1,2,3</sup> John H. Postlethwait,<sup>6</sup> J. Robert Manak,<sup>10</sup> Eric M. Thompson,<sup>4,19</sup> Olivier Jaillon,<sup>1,2,3</sup> Louis Du Pasquier,<sup>20</sup> Pierre Boudinot,<sup>21</sup> David A. Liberles,<sup>22</sup> Jean-Nicolas Volf,<sup>23</sup> Hervé Philippe,<sup>5</sup> Boris Lenhard,<sup>4,7,19</sup> Hugues Roest Crollius,<sup>9</sup> Patrick Wincker,<sup>1,2,3,†</sup> Daniel Chourrout<sup>4,†</sup>

Genomes of animals as different as sponges and humans show conservation of global architecture. Here we show that multiple genomic features including transposon diversity, developmental gene repertoire, physical gene order, and intron-exon organization are shattered in the tunicate *Oikopleura*, belonging to the sister group of vertebrates and retaining chordate morphology. Ancestral architecture of animal genomes can be deeply modified and may therefore be largely nonadaptive. This rapidly evolving animal lineage thus offers unique perspectives on the level of genome plasticity. It also illuminates issues as fundamental as the mechanisms of intron gain.

Tunicates, viewed as the closest living relatives of vertebrates, were probably simplified from more complex chordate ancestors (*1*). Larvacean tunicates represent the second most abundant component of marine zooplankton and filter small particles by their gelatinous house. *Oikopleura dioica* is the most cosmopolitan larvacean, has a very short life cycle (4 days at 20°C), and can be reared in the laboratory for hundreds of

generations (*2*). Unique among tunicates, it has separate sexes. We sequenced its genome with high-coverage shotgun reads (14X) using males resulting from 11 successive full-sib matings (figs. S1 and S2 and tables S1 to S3) (*3*). Two distinct haplotypes were retained, despite inbreeding. Their comparison yielded a high estimate of population mutation rate ( $\theta = 4N_e\mu = 0.0220$ ) that is consistent with a large effective population size ( $N_e$ ) and/or a

high mutation rate per generation ( $\mu$ ) (*3*). Sequence comparisons among populations from the eastern Pacific and eastern Atlantic and within the latter revealed low  $dN/dS$  values ( $dN$ , rate of substitutions at nonsilent sites;  $dS$ , rate of substitutions at silent sites) consistent with strong purifying selection, as expected for large populations (*3*). In 17 of 18

<sup>1</sup>Commissariat à l'Énergie Atomique, Institut de Génomique, Genoscope, Evry, France. <sup>2</sup>CNRS, UMR 8030, Evry, France. <sup>3</sup>Université d'Evry, Evry, France. <sup>4</sup>Sars International Centre for Marine Molecular Biology, University of Bergen, Bergen, Norway. <sup>5</sup>Département de Biochimie, Université de Montréal, Montréal, Canada. <sup>6</sup>Institute of Neuroscience, University of Oregon, Eugene, OR 97403, USA. <sup>7</sup>Bergen Center for Computational Science, University of Bergen, Bergen, Norway. <sup>8</sup>Laboratorio de Genética, Universidad de Cádiz, Cádiz, Spain. <sup>9</sup>Dyogen Lab, Institut de Biologie de l'ENS (IBENS), CNRS-UMR8197, Ecole Normale Supérieure, Paris, France. <sup>10</sup>Department of Biology, University of Iowa, Iowa City, IA 52242-1324, USA. <sup>11</sup>Laboratoire de Paléontologie, Phylogénie et Paléobiologie, Institut des Sciences de l'Évolution, UMR 5554-CNRS, Université Montpellier II, Montpellier, France. <sup>12</sup>Department of Statistics, University of Wyoming, Laramie, WY 82071, USA. <sup>13</sup>Institut de Biologie Cellulaire et Moléculaire du CNRS, Université de Strasbourg, Strasbourg, France. <sup>14</sup>Department of Biological Sciences, Osaka University, Osaka, Japan. <sup>15</sup>Centre Hospitalier d'Albi, Albi, France. <sup>16</sup>Department of Molecular Biology, University of Bergen, Bergen, Norway. <sup>17</sup>Vertebrate Genomics, Max Planck Institute for Molecular Genetics, Berlin, Germany. <sup>18</sup>National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD 20894, USA. <sup>19</sup>Department of Biology, University of Bergen, Bergen, Norway. <sup>20</sup>Institute of Zoology and Evolutionary Biology, University of Basel, Basel, Switzerland. <sup>21</sup>Institut National de la Recherche Agronomique (INRA), Virologie et Immunologie Moléculaires, Jouy-en-Josas, France. <sup>22</sup>Department of Molecular Biology, University of Wyoming, Laramie, WY 82071, USA. <sup>23</sup>Institut de Génomique Fonctionnelle de Lyon, UMR 5242-CNRS/INRA/Université Claude Bernard Lyon 1/Ecole Normale Supérieure, Ecole Normale Supérieure de Lyon, Lyon, France. <sup>24</sup>Département de Genética, Universitat de Barcelona, Spain.

\*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: Daniel.Chourrout@sars.uib.no (D.C.); pwincker@genoscope.cns.fr (P.W.)

phylogenetic trees constructed with 26 metazoan genomes and nine independent data sets, *Oikopleura* shows the fastest protein evolution, and even higher evolutionary rates are observed for mitochondrial genes that are heavily modified by oligo-dT insertions (figs. S3 to S6 and tables S4 to S6) (3). Key components of DNA repair, especially in the nonhomologous end-joining pathway, were not detected in the genome (fig. S7 and table S7) (3). Coincident rapid evolution of nuclear and mitochondrial genomes may also reflect a highly mutagenic context at the ocean surface.

At 70 megabases with 18,020 predicted genes, the *Oikopleura* genome is unusually compact. Introns are very small (peak at 47 base pairs, 2.4% > 1 kb), as are intergenic spaces, partly because of numerous operons (fig. S8 and table S8) (3). Genes outside operons are also densely packed (53% of intergenic distances < 1 kb). Even compared with other compact genomes (4), the density of transposable elements (TEs) is low. Most pan-animal TE superfamilies are absent in *Oikopleura*, and only two species-specific clades of retrotransposons (5) have diversified. A massive purge of ancient TEs can be invoked, but TEs currently present in the genome show multiple signs of activity (figs. S9 to S16) (3). The low copy number of each element and the uneven genome distribution of the main TE clades suggest tight control of their proliferation (Fig. 1A) (3).

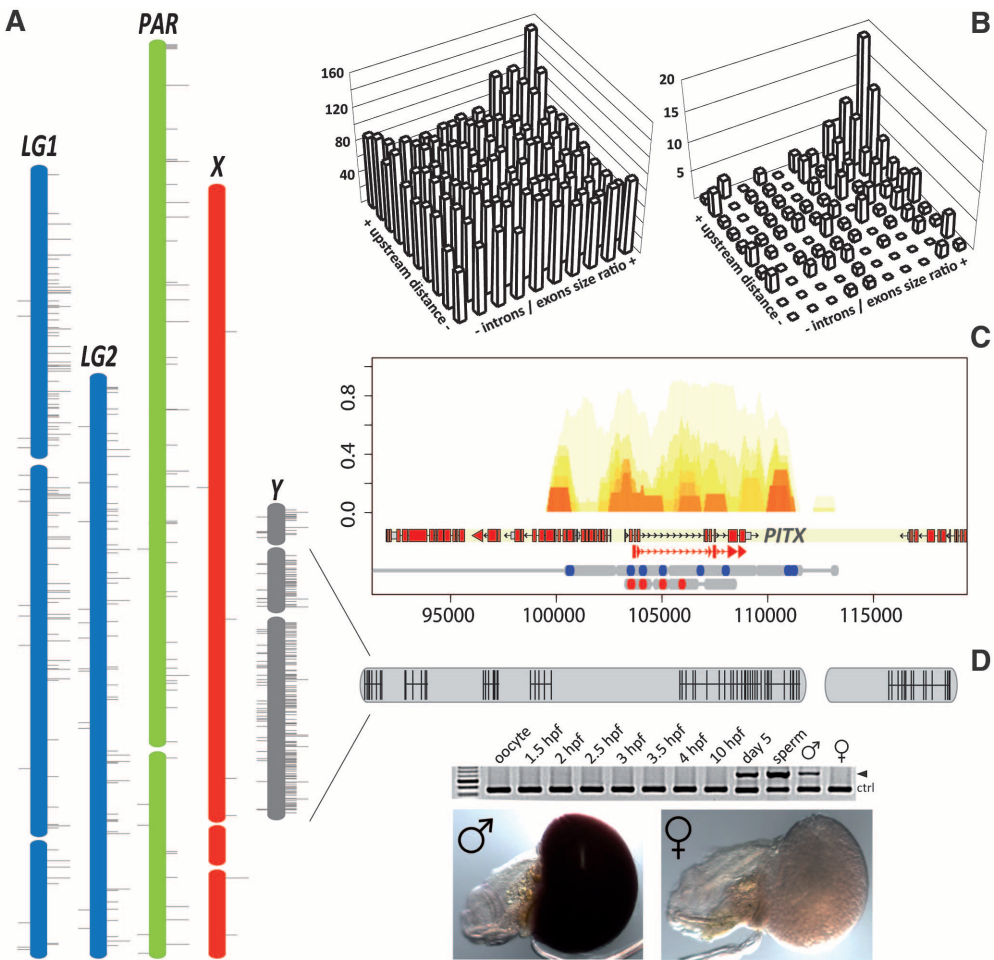
Two exceptions to global compaction are particularly interesting, as they may illustrate where excessive reduction is harmful. First, a small pop-

ulation of *Oikopleura* genes has relatively large introns and intergenic spaces (Fig. 1B). It is enriched for developmentally regulated transcription

**Table 1.** Minimal immune system predicted from the *Oikopleura* genome. Numbers of genes or domains in families encoding potential immunity factors. *D.m.*, *Drosophila melanogaster*; *S.p.*, *Strongylocentrotus purpuratus*; *O.d.*, *Oikopleura dioica*; *C.i.*, *Ciona intestinalis*; *B.f.*, *Branchiostoma floridae*; *P.m.*, *Petromyzon marinus*; *H.s.*, *Homo sapiens*. TLR, Toll-like receptor; NLR, NOD-like receptor; SRCR, scavenger receptor cysteine-rich; PGRP, peptidoglycan recognition protein; RIG-I, retinoic acid-inducible gene-I; IgSF-ITIM, immunoglobulin superfamily domain with immunoreceptor tyrosine inhibitory motif; DEATH-TIR, DEATH superfamily members with Toll/interleukin-1-receptor domain; SARM1, SAM- and ARM-containing protein 1; TIRAP, Toll/interleukin-1-receptor domain-containing adapter protein; TICAM2, Toll/interleukin-1-receptor domain-containing adapter molecule; PLA2, phospholipase A2. ND, not determined.

|                                     | <i>D.m.</i> | <i>S.p.</i> | <i>O.d.</i> | <i>C.i.</i> | <i>B.f.</i> | <i>P.m.</i> | <i>H.s.</i> |
|-------------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| <i>Sensors</i>                      |             |             |             |             |             |             |             |
| TLR                                 | 9           | 222         | 1           | 3           | 48          | 21          | 10          |
| NLR                                 | 0           | 203         | 0           | 20          | 92          | 140–220     | 20          |
| SRCR                                | 14          | 218         | 1           | 81          | 270         | 287         | 81          |
| PGRP                                | 15          | 5           | 4           | 6           | >20         | ND          | 6           |
| RIG-I-like helicases                | 0           | 12          | 0           | ND          | 7           | ND          | 3           |
| C-type lectins                      | 32          | 104         | 31          | 120         | 1215        | ND          | 81          |
| IgSF-ITIM                           | >3          | ND          | 5           | >6          | >5          | >3          | >50         |
| <i>Adaptors</i>                     |             |             |             |             |             |             |             |
| MyD88-like (DEATH-TIR)              | 1           | 4           | 0           | 1           | 4           | ND          | 1           |
| SARM1-like, TIRAP-like, TICAM2-like | 1           | 15          | 0           | >2          | 12          | ND          | 3           |
| <i>Potential effector</i>           |             |             |             |             |             |             |             |
| PLA2                                | 8           | 65          | 128         | 7           | >7          | ND          | 11          |

**Fig. 1.** Genome compaction features. (A) Chromosome regions assembled with physical links and genetic markers. The location of TEs is indicated with horizontal lines (lines on the left sides, DNA transposons; lines on right sides, short lines for long terminal repeat–retrotransposons and long lines for long interspersed elements). (B) Distribution of gene models over 10% abundance classes of intron size and upstream intergenic distance for 8812 nonoperon genes (left) and for 189 developmentally regulated genes, mainly transcription factors (right). (C) Conserved elements revealed in genome alignments of Atlantic and Pacific ocean populations of *O. dioica*: density of conserved blocks (top), gene annotation (middle), and perfectly conserved elements >100 bp (bottom gray line) (blue, Norway versus northwest America; red, Norway versus Japan). (D) Giant Y genes and their testis expression revealed by reverse transcription polymerase chain reaction and in situ hybridization. hpf, hours post fertilization; ctrl, control. The arrowhead indicates the giant gene expression product.



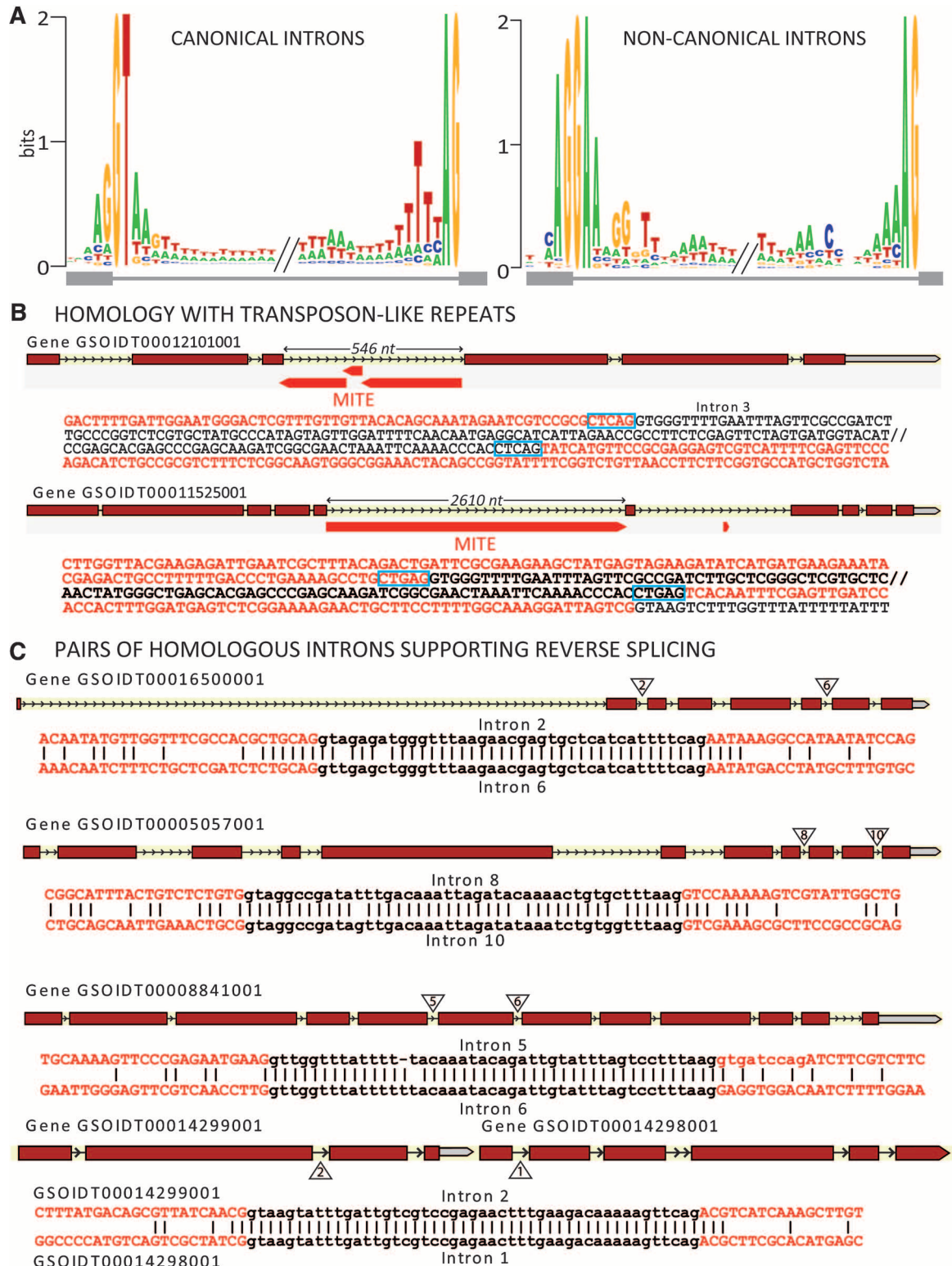


factor genes that are long in other genomes because of an abundance of regulatory elements (6). Regulatory-element sequences can be highly conserved, though rarely across phyla, and *Oikopleura* homologs of vertebrate conserved elements were not detected (3). However, a comparison of genes encoding developmental transcription factors from Atlantic and Pacific *O. dioica* revealed short seg-

ments of higher sequence conservation in non-coding regions than in exons, suggestive of a rich regulatory content (Fig. 1C and fig. S17) (3). Interestingly, in a revolution of massive intron loss (see below), *Oikopleura* retained large introns more often than small ones, and the ratio of ancestral to newly acquired introns is highest in developmental transcription factor genes (figs. S18 and S19) (3).

Second, Mendelian analysis showed that sex in *Oikopleura* is genetically determined (fig. S20 and table S10) (3), and we could reconstruct large X and Y chromosomes (Fig. 1A). Seven genes on the Y chromosome, all expressed in the testis during spermatogenesis, have giant introns (Fig. 1D). Their size probably grew with the nonrecombining Y chromosome region, flaunting global compaction.

**Fig. 2.** Introns and intron gain scenarios. (A) Main intron logos. (B) Transposon insertion: Duplicated insertion sites (framed in blue) allow miniature inverted repeat (MITE)-like insertions to be spliced out exactly (red, exons; black, introns). (C) Reverse splicing: four pairs of homologous introns (black) and their immediate exonic environments (red).

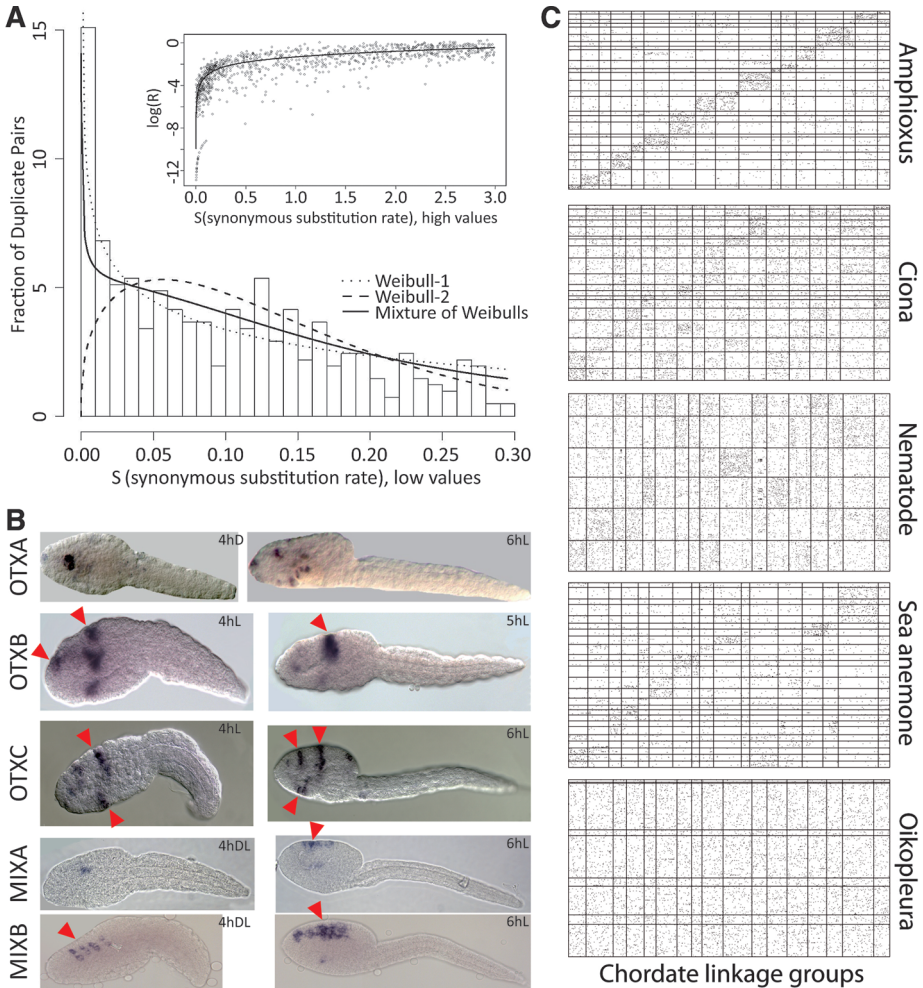


*Oikopleura* has a rather common number of introns per gene (4.1), but the turnover of its introns has been extraordinarily high: Of 5589 introns mapped by interspecies protein alignments, 76% had positions unique to *Oikopleura* (newly acquired introns), 17% were at ancestral positions (old introns), and 7% could not be classified (fig. S21) (3). Non-canonical introns, mostly GA-AG and with a very specific acceptor site, are unusually frequent (12%) (Fig. 2A and figs. S22 to S25) (3). They show several peculiarities (tables S11 and S12), including preferential insertion in phase 1, which is compatible with the current codon usage, as would be expected for the most recently gained introns (3, 7). The most distinctive feature of newly acquired introns (figs. S26 and S27 and tables S13 to S15) is that they are more often noncanonical than old introns (8.4 versus 2.6%) (3). Because *Oikopleura* lacks the minor spliceosome and has only one type of each spliceosomal component, we propose that a single and permissive major spliceosome is used, with U1snRNP (where snRNP is small nuclear ribonucleoprotein) and U2AF able to recognize donor and acceptor sites (3, 8, 9). cDNA sequence information suggests an efficient splicing for the vast majority of introns. A permissive spliceosome could favor intron gains by correctly splicing out newly acquired introns. The pattern

of intron loss in *Oikopleura* is consistent with homologous recombination of reverse transcribed mRNA (table S16) (3, 10). Among hypothetical mechanisms of intron gain, we provide evidence for the insertion of transposon-like elements and, more remarkably for reverse splicing, a reaction in which spliced out introns can be ectopically reinserted into transcripts (11). We identified 32 compelling candidate introns for transposon insertion (Fig. 2B and table S17) (3), those matching repetitive elements containing terminal repeats at almost all nucleotides, with exons excluded. These introns were usually hemizygous in genotyped individuals, but one individual was homozygous and displayed spliced transcripts (figs. S28 to S30 and table S18) (3). We also identified four pairs of nearly identical introns (NIIs) with no or very weak similarity in flanking exons (Fig. 2C) (3), which, to the best of our knowledge, represent the first reported candidates for reverse splicing (12). All animals were homozygous for NIIs and had spliced transcripts (fig. S31 and table S19) (3). Notably, introns of each pair of NIIs were found within the same gene or the same operon, suggesting intron propagation within their pre-mRNA. Many newly acquired introns of *Oikopleura* might have been propagated like these four NIIs before their sequences diverged, because they tend to be adjacent

in their host gene (table S20) (3). Competing mechanisms remain possible: First, introns could be reverse spliced into the genome itself, as can group II introns (13). Some, and possibly many, introns of *Oikopleura* could originate by repair of double strand breaks (DSBs), as proposed for newly acquired introns in *Daphnia* (14). However, for the four mentioned intron pairs, a repair after a DSB would not readily explain the systematic colocalization of homologous introns in the same transcription unit. No feature in the sequences of those introns in pairs and their surroundings brings particular support for this mechanism (3). We explored the *Oikopleura* genome for genes involved in either development or immunity. Many conserved immunity genes failed detection, supporting a minimized immune system consistent with the short *Oikopleura* life history (Table 1 and table S21) (3). Although frequent gene losses may have affected families of developmental genes, we were most intrigued by an unusually large number of lineage-specific duplicates, thus far reported for homeobox genes only (15): 87 amplifications accounting for 266 current genes (table S22) (3), versus 40 amplifications in *Ciona* giving 106 current genes (16). A survival analysis of early duplicates in the genome showed that duplicates are initially lost very rapidly with less relaxed selec-

**Fig. 3.** Gene duplications and loss of ancestral synteny. **(A)** Early gene duplicates. (Main panel) Histogram of binned recent duplicate pairs; a mixture model (discrete distribution plus truncated Weibull distributions) accommodating heterogeneous birth/death processes is fitted. (Inset) Non-synonymous substitution accumulation declines with ongoing synonymous substitution. **(B)** Expression of amplified homeobox gene groups in the trunk epithelium of larvae (red arrowheads). hD, hours dorsal view; hL, hours lateral view; hDL, hours dorso-lateral view. **(C)** Loss of ancestral gene order. Positions of orthologous genes in a given metazoan genome (y axis) compared with ancestral chordate linkage groups [(CLGs), x axis]. The width of CLGs corresponds to the number of orthologs in a given species. Amphioxus and sea anemone genome segments represent the largest 25 assembled scaffolds, whereas *Ciona*, nematode, and *Oikopleura* segments are chromosomes.





tion than in mammalian genomes (17). In contrast, those that survive beyond 0.02 dS units are relatively more likely to be retained (Fig. 3A, figs. S32 to S34, and table S23) (3). To understand how older developmental gene duplicates are used, we focused on homeobox genes. Notably, we detected broad expression signals in the larval trunk epithelium for genes of most amplified groups (16 in 20), but rarely for other groups (1 in 19) (Fig. 3B, fig. S35, and table S24), likely reflecting roles in patterning of the house-building epithelium (18), a crucial novelty of larvaceans. A preferential retention of duplicates for developmental genes has occurred in vertebrates after whole-genome duplications. Their massive retention in *Oikopleura* is exceptional among invertebrates. In addition to neofunctionalization for complex innovations like house production, another explanation may take into consideration the general reduction of gene size in *Oikopleura*. This may enhance the likelihood for developmental genes to escape truncation after the local rearrangements that cause duplications (19). Other mechanisms may facilitate duplications or preserve developmental gene duplicates in *Oikopleura*.

Finally, we compared synteny relationships in *Oikopleura* and several invertebrates to ancestral chordate linkage groups (3, 20). Amphioxus, *Ciona*, *Caenorhabditis*, and sea anemone showed many cases of conserved chromosomal synteny (Fig. 3C, figs. S36 and S37, and table S25), but *Oikopleura* orthologs showed no such conservation. We also measured local synteny conservation between the same species and human (3). Amphioxus, *Ciona*, *Caenorhabditis*, and sea anemone (to a much lower degree) displayed significantly higher conservation of neighborhood than expected by chance. *Oiko-*

*pleura* showed a local gene order that is indistinguishable from random for distances smaller than 30 genes and a modest level of conserved synteny at larger distances (fig. S38).

We show that multiple genome-organization features, conserved across metazoans including other tunicates and nonbilaterians, have dramatically changed in the *Oikopleura* lineage. Despite an unprecedented genome revolution, the *Oikopleura* lineage preserved essential morphological features, even maintaining the chordate body plan to the adult stage, unlike other tunicates. Evolution in this lineage was rapid and probably took place in a context favoring purifying selection against mildly deleterious features. Our results strengthen the view that global similarities of genome architecture from sponges to humans (20–23) are not essential for the preservation of ancestral morphologies, as is widely believed (24–26).

### References and Notes

1. F. Delsuc, H. Brinkmann, D. Chourrout, H. Philippe, *Nature* **439**, 965 (2006).
2. J. M. Bouquet et al., *J. Plankton Res.* **31**, 359 (2009).
3. Supporting methods and results are available on Science Online.
4. T. H. Eickbush, A. V. Furano, *Curr. Opin. Genet. Dev.* **12**, 669 (2002).
5. J. N. Volff, H. Lehrach, R. Reinhardt, D. Chourrout, *Mol. Biol. Evol.* **21**, 2022 (2004).
6. A. Woolfe et al., *PLoS Biol.* **3**, e7 (2005).
7. H. D. Nguyen, M. Yoshihama, N. Kenmochi, *BMC Evol. Biol.* **6**, 69 (2006).
8. M. Marz, T. Kirsten, P. F. Stadler, *J. Mol. Evol.* **67**, 594 (2008).
9. D. A. R. Zorio, T. Blumenthal, *Nature* **402**, 835 (1999).
10. T. Mourier, D. C. Jeffares, *Science* **300**, 1393 (2003).
11. S. W. Roy, W. Gilbert, *Nat. Rev. Genet.* **7**, 211 (2006).
12. S. W. Roy, M. Irimia, *Trends Genet.* **25**, 67 (2009).

13. B. Cousineau et al., *Cell* **94**, 451 (1998).
14. W. Li, A. E. Tucker, W. Sung, W. K. Thomas, M. Lynch, *Science* **326**, 1260 (2009).
15. R. B. Edvardson et al., *Curr. Biol.* **15**, R12 (2005).
16. Y. Satou, N. Satoh, *Dev. Genes Evol.* **213**, 211 (2003).
17. T. Hughes, D. A. Liberles, *J. Mol. Evol.* **65**, 574 (2007).
18. E. M. Thompson, T. Kalløe, F. Spada, *Dev. Biol.* **238**, 260 (2001).
19. V. Katju, M. Lynch, *Genetics* **165**, 1793 (2003).
20. N. H. Putnam et al., *Nature* **453**, 1064 (2008).
21. N. H. Putnam et al., *Science* **317**, 86 (2007).
22. M. Srivastava et al., *Nature* **466**, 720 (2010).
23. M. Srivastava et al., *Nature* **454**, 955 (2008).
24. M. Lynch, J. S. Conery, *Science* **302**, 1401 (2003).
25. M. Lynch, *Mol. Biol. Evol.* **23**, 450 (2006).
26. M. Lynch, *Proc. Natl. Acad. Sci. U.S.A.* **104** (suppl. 1), 8597 (2007).
27. The Sars Centre budget, the Functional Genomics (FUGE) Programme of the Norwegian Research Council, Genoscope, and NSF grants IOS-0719577 and DBI-0743374 supported the research. This publication ISEM-2010-123 of the Institut des Sciences de l'Évolution de Montpellier. GENBANK/European Molecular Biology Laboratory sequence accession numbers are CABV01000001-CABV01005917, CABW01000001-CABW01006678, FN653015-FN654274, FN654275-FN658470, FP700189-FP710243, FP710258-FP791398, and FP791400-FP884219. The sequence data for *Capitella teleta*, *Daphnia pulex*, *Helobdella robusta* and *Lottia gigantea* were produced by the U.S. Department of Energy Joint Genome Institute ([www.jgi.doe.gov/](http://www.jgi.doe.gov/)) in collaboration with the community of users. We thank I. Ahel, B. Haubold, and one anonymous reviewer for generous advice. This article is dedicated to Hans Prydz and Kåre Rømmetveit for their pioneer roles in the Sars Centre establishment.

### Supporting Online Material

[www.sciencemag.org/cgi/content/full/science.1194167/DC1](http://www.sciencemag.org/cgi/content/full/science.1194167/DC1)  
Methods  
SOM Text  
Figs. S1 to S38  
Tables S1 to S26  
References

24 June 2010; accepted 29 October 2010  
10.1126/science.1194167

## Rewiring of Genetic Networks in Response to DNA Damage

Sourav Bandyopadhyay,<sup>1</sup> Monika Mehta,<sup>2</sup> Dwight Kuo,<sup>3</sup> Min-Kyung Sung,<sup>4</sup> Ryan Chuang,<sup>3</sup> Eric J. Jaehnig,<sup>5</sup> Bernd Bodenmiller,<sup>6</sup> Katherine Licon,<sup>1</sup> Wilbert Copeland,<sup>3</sup> Michael Shales,<sup>7</sup> Dorothea Fiedler,<sup>7,8</sup> Janusz Dutkowski,<sup>1</sup> Aude Guénolé,<sup>9</sup> Haico van Attikum,<sup>9</sup> Kevan M. Shokat,<sup>7,8</sup> Richard D. Kolodner,<sup>5,1,10</sup> Won-Ki Huh,<sup>4</sup> Ruedi Aebersold,<sup>6</sup> Michael-Christopher Keogh,<sup>2\*</sup> Nevan J. Krogan,<sup>7\*</sup> Trey Ideker<sup>1,3,10\*</sup>

Although cellular behaviors are dynamic, the networks that govern these behaviors have been mapped primarily as static snapshots. Using an approach called differential epistasis mapping, we have discovered widespread changes in genetic interaction among yeast kinases, phosphatases, and transcription factors as the cell responds to DNA damage. Differential interactions uncover many gene functions that go undetected in static conditions. They are very effective at identifying DNA repair pathways, highlighting new damage-dependent roles for the Slr2 kinase, Pph3 phosphatase, and histone variant Htz1. The data also reveal that protein complexes are generally stable in response to perturbation, but the functional relations between these complexes are substantially reorganized. Differential networks chart a new type of genetic landscape that is invaluable for mapping cellular responses to stimuli.

One of the most basic approaches to understanding gene function relies on the identification of genetic interactions, which

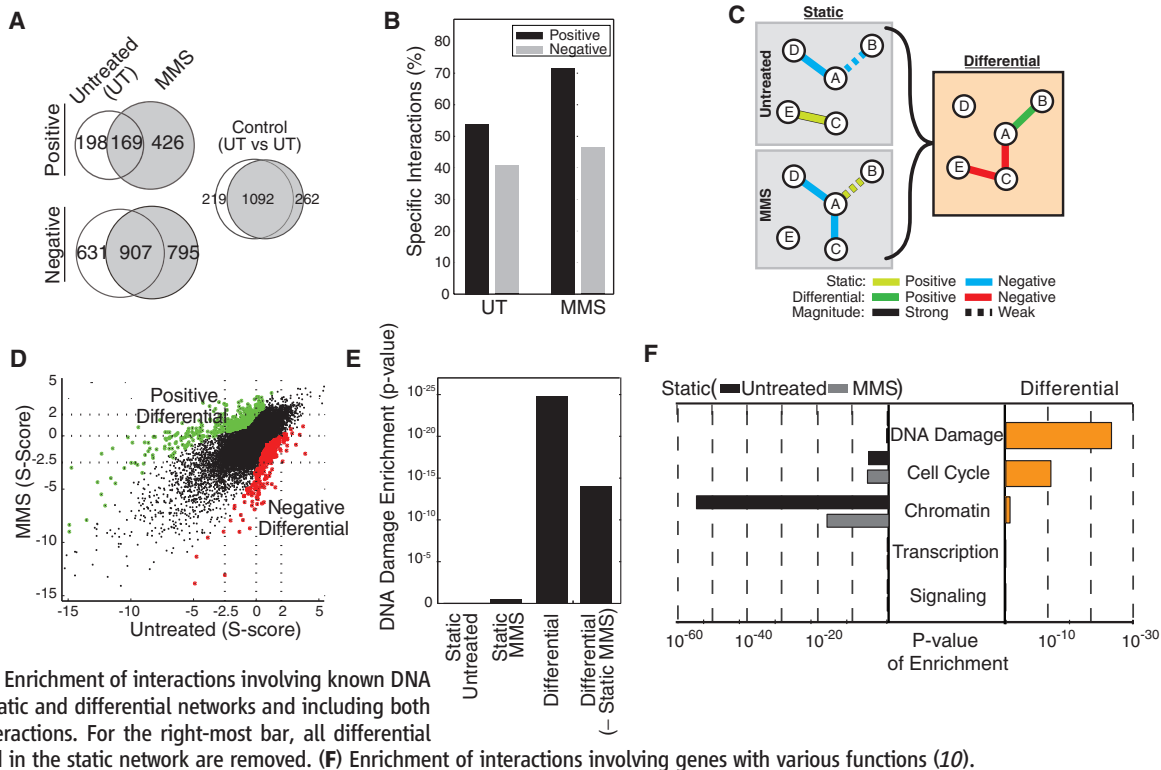
occur when the phenotypic effects of one gene depend on the presence of a second. Recently, a number of technologies have been developed to

systematically map genetic interaction networks over large sets of genes in budding yeast (1–3) and other model organisms (4, 5). Thus far, these networks have been constructed only under normal laboratory conditions. However, cells are constantly bombarded by signals and stresses, such as ligands, drugs, hormones, toxins, or other

<sup>1</sup>Department of Medicine, University of California, San Diego, La Jolla, CA 92093, USA. <sup>2</sup>Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461, USA. <sup>3</sup>Department of Bioengineering, University of California, San Diego, La Jolla, CA 92093, USA. <sup>4</sup>School of Biological Sciences and Research Center for Functional Cellulomics, Institute of Microbiology, Seoul National University, 151-742 Seoul, Republic of Korea. <sup>5</sup>Ludwig Institute for Cancer Research and Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92093, USA. <sup>6</sup>Institute of Molecular Systems Biology, ETH Zürich, Zürich CH 8093, Switzerland, and Faculty of Science, University of Zürich, Zürich CH 8057, Switzerland. <sup>7</sup>Department of Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, CA 94158, USA. <sup>8</sup>Howard Hughes Medical Institute, San Francisco, CA 94158, USA. <sup>9</sup>Department of Toxicogenetics, Leiden University Medical Center, Leiden, Netherlands. <sup>10</sup>The Institute for Genomic Medicine, University of California, San Diego, La Jolla, CA 92093, USA.

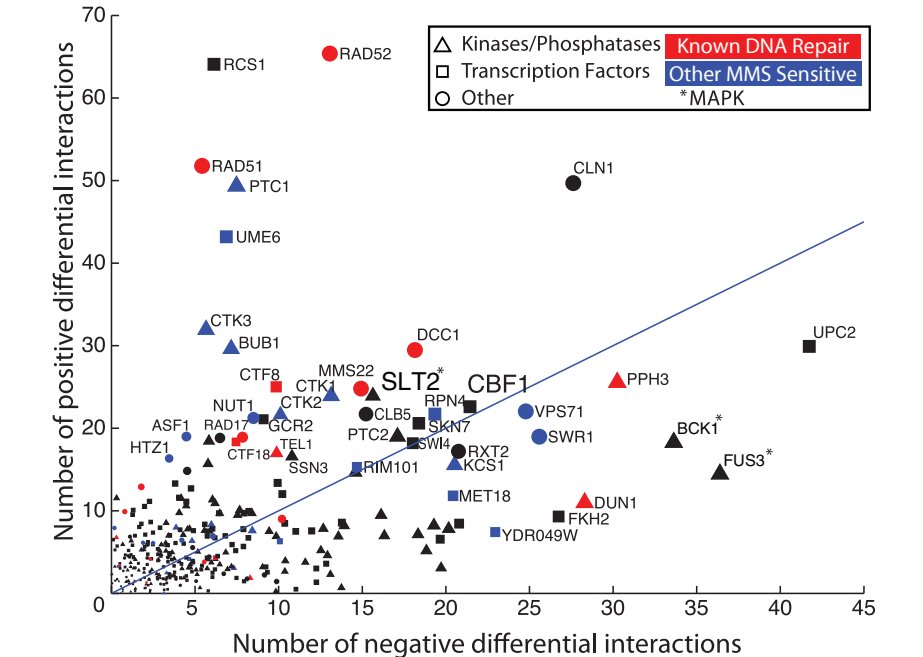
\*To whom correspondence should be addressed. E-mail: [tideker@ucsd.edu](mailto:tideker@ucsd.edu) (T.I.); [krogan@comp.ucsf.edu](mailto:krogan@comp.ucsf.edu) (N.J.K.); [michael.keogh@einstein.yu.edu](mailto:michael.keogh@einstein.yu.edu) (M.-C.K.)

**Fig. 1.** An epistasis map for DNA damage signaling. **(A)** Comparison of genetic interactions (positive,  $S \geq +2$ ; negative,  $S \leq -2.5$ ) uncovered in untreated or DNA damage treated (+MMS) conditions. Control represents interactions from two independent experiments in untreated conditions. **(B)** Percentage of interactions (positive or negative) identified in each condition that are specific to that condition. **(C)** Differences between the untreated and treated maps identify differential interactions. **(D)** Scatter of S scores between untreated and treated maps and identification of positive differential (green,  $P \leq 0.001$ ) and negative differential interactions (red,  $P \leq 0.001$ ). **(E)** Enrichment of interactions involving known DNA repair genes, shown for static and differential networks and including both positive and negative interactions. For the right-most bar, all differential interactions also identified in the static network are removed. **(F)** Enrichment of interactions involving genes with various functions (10).



environmental conditions. Although it is clear that some genetic interactions are condition-dependent (6, 7), to what extent environmental stresses can affect genetic interaction networks, and the pathways they represent, is still unknown. To gain insight into how genetic networks are altered by stress, we assembled a large genetic interactome with and without perturbation by the DNA-damaging agent methyl methane-sulfonate (MMS). Using the technique of epistatic miniarray profiles (E-MAP) (8), genetic interactions were interrogated among a set of 418 yeast genes selected to provide broad coverage of the cellular signaling and transcriptional machinery, including nearly all yeast kinases, phosphatases, and transcription factors, as well as known DNA repair factors (fig. S1 and table S1). About 80,000 double-mutant strains were generated from all pairwise mutant combinations of the 418 genes, in which mutations were complete gene deletions (nonessential genes) or hypomorphic alleles (essential genes) as appropriate. Double-mutant combinations were grown with or without 0.02% MMS, and their colony sizes were analyzed statistically to compute a genetic interaction score (S score) in each condition (9), which indicates whether the strain was healthier or sicker than expected (positive or negative S, respectively) (10).

From established score thresholds for positive and negative interactions ( $S \geq +2.0$ ,  $S \leq -2.5$ ) (9) we identified two genetic networks: a set of 1905 interactions for the untreated condition, and a set of 2297 interactions under MMS. Analysis of these “static” genetic maps showed strong asso-



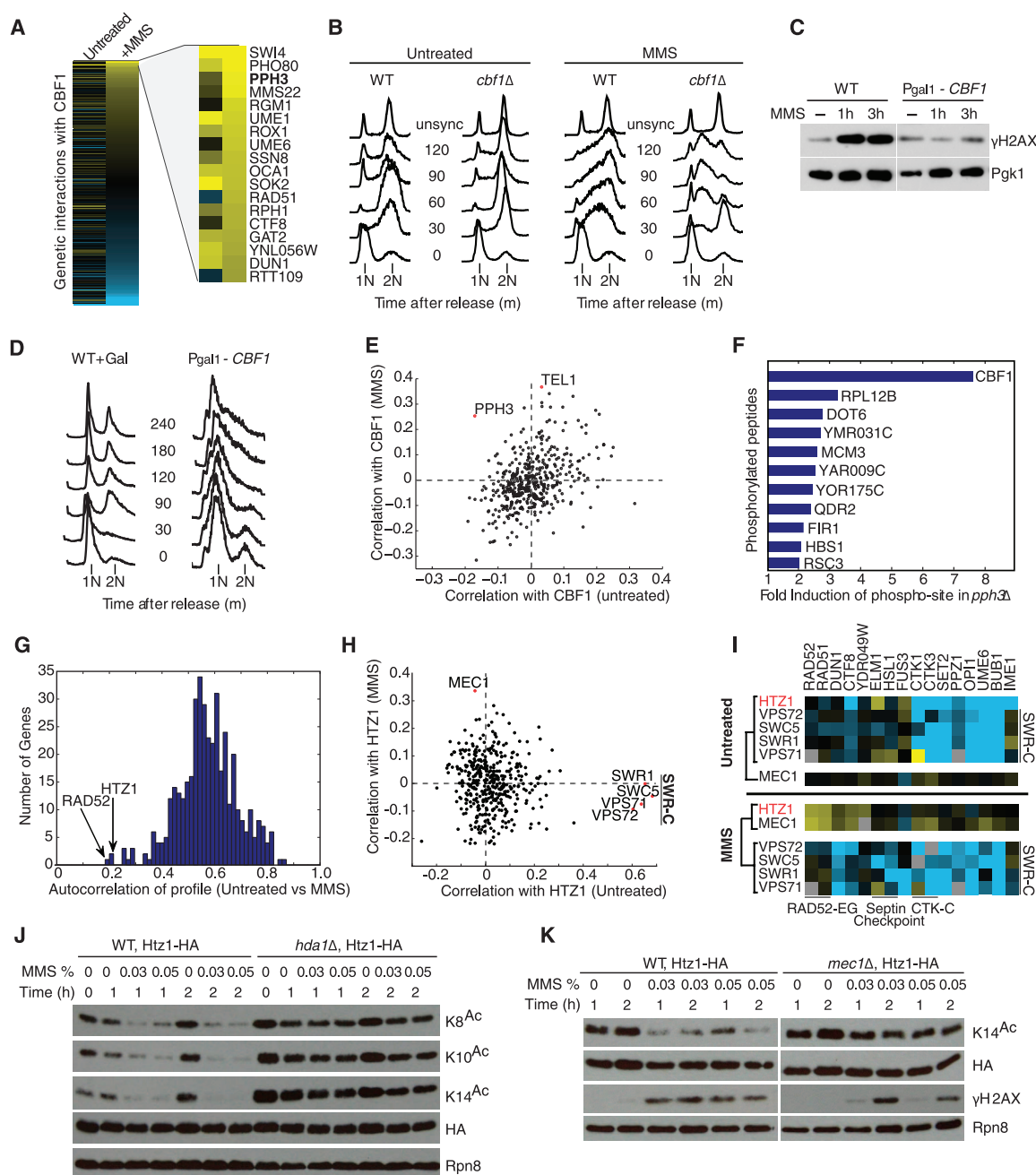
**Fig. 2.** Identification of differential genetic interaction hubs. The scatterplot shows the number of positive and negative differential interactions associated with each gene in this study. The 30 genes whose deletions are the most sensitive to MMS are indicated (blue) (24), excluding those already known to function in DNA repair (red) (table S1).

ciations with physical interaction networks of various kinds. For example, gene pairs with either positive or negative genetic interactions were highly enriched for proteins known to physically interact. In addition, both maps were enriched for known

kinase- and phosphatase-substrate pairs, as well as transcription factor-target pairs (fig. S2). The correspondence to physical and functional associations reflects the predictive power of this genetic interaction data set.



**Fig. 3.** Differential genetic interactions identify novel DNA damage-dependent pathways. **(A)** Full profile of *CBF1* genetic interactions with the strongest positive genetic interactions in MMS highlighted. **(B)** Fluorescence-activated cell sorting (FACS) analysis of cell cycle progression in alpha-factor-arrested wild-type and *cbf1Δ* cells released into media with or without MMS. In MMS, *cbf1Δ* cells bypass cell cycle checkpoints and eventually accumulate in S phase (between 1N and 2N DNA content). **(C)**  $\gamma$ H2AX levels for wild-type and *CBF1* overexpression at times indicated after exposure to MMS. Pgk1 is used as a loading control. **(D)** Effect of *CBF1* overexpression on cell cycle progression using FACS. **(E)** Correlation coefficients between the *CBF1* genetic interaction profile and that of each gene in the epistasis map, in MMS versus untreated conditions. **(F)** Top changes in abundance of phosphorylated peptides in *pph3Δ* versus wild-type cells by phospho-proteomic profiling. **(G)** Histogram of autocorrelation coefficients. For each gene, the genetic interaction profiles before and after MMS exposure are compared by Pearson correlation. **(H)** Correlation plot as in (E) for *HTZ1*. **(I)** Representative genetic interactions of *HTZ1*, *MEC1*, and *SWR-C* members. Clustering based on similarity of profiles is shown. **(J)** The acetylation of multiple Htz1 N-terminal lysine residues (K8, K10, or K14) measured in an Htz1-3HA strain after MMS exposure in wild-type (WT) and *hda1Δ* backgrounds. HA represents total



Comparison of the genetic networks across conditions revealed large differences, with more interactions unique to each map than in common (Fig. 1A). For example, more than 70% of positive interactions identified under MMS were not identified in the untreated sample, which reflects widespread DNA damage-induced epistasis (Fig. 1B). To assess these changes in interaction, each gene pair was associated with its difference in S score across conditions (Fig. 1C). A *P* value for this difference was calculated using the null distribution of score differences observed when comparing replicate interaction measurements from the same

condition (fig. S3) (10). This method identified 873 differential genetic interactions at  $P \leq 0.001$ , with a corresponding false-discovery rate of ~9% (fig. S4 and table S2). We term this approach differential epistasis mapping (dE-MAP), as it is based on the difference of two static networks generated using the E-MAP methodology. A total of 379 interactions were “negative differential,” which indicated DNA damage-induced lethality or sickness, whereas 494 were “positive differential,” which indicated inducible epistasis or suppression (Fig. 1D). The majority (62%) of differential interactions were not detectable in

either static condition, most likely because they are too weak to detect in any single condition yet display a substantial change in interaction between conditions.

To determine whether static untreated, static treated, or differential genetic networks best uncover DNA damage-response pathways, we examined a reference set of 31 known DNA repair genes (table S1). We noted that static networks were no more likely than random to include interactions with genes in this reference set (Fig. 1E). This lack of enrichment was observed in the untreated genetic network, as well as, sur-

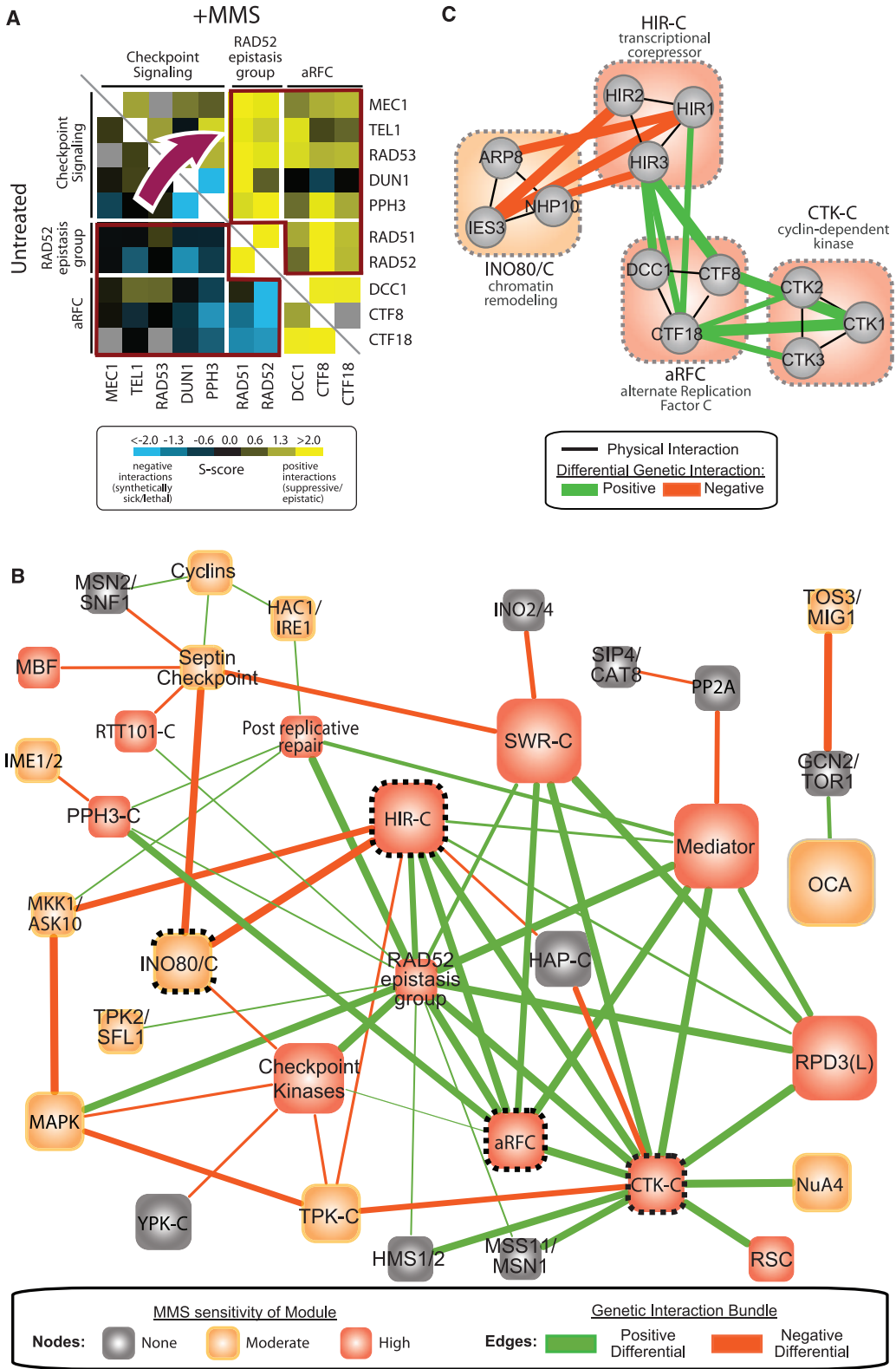
amount of Htz1. Rpn8 is a loading control. **(K)** Acetylation status of Htz1-K14 in response to MMS in WT and *mec1Δ* backgrounds.  $\gamma$ H2AX is a downstream marker of DNA-damage signaling by Mec1.

prisingly, the static network obtained under MMS. In contrast, the differential network—obtained through the quantitative difference in interaction across conditions—was highly enriched for interactions involving DNA damage-response genes such as *RAD52*, *TEL1*, and *DUN1* (Fig. 1E) (10).

We noted that both the static treated and untreated networks were dominated by interactions involving genes that function in chromatin organization (Fig. 1F). This strong chromatin signal has been previously reported in budding and fission yeasts and *C. elegans* (2, 4, 5, 11). Through

network subtraction, however, the “housekeeping interactions” due to chromatin are removed, which allows sensitive detection of differentially represented pathways. Thus, network comparison reveals a landscape of genetic interactions particularly tailored to the cellular response of interest.

**Fig. 4. Module-based interpretation of differential genetic interactions.** (A) MMS induces a set of positive interactions between DNA damage pathways (top right) that are not evident in untreated conditions (bottom left). (B) Module map of protein complexes and pathways connected by differential genetic interaction bundles. Node color represents the most severe single-deletion phenotype among members of a module (table S1). (C) Detailed view of differential genetic interactions between protein complexes corresponding to selected modules in (B) (dotted node borders). For clarity, only physical interactions and differential genetic interactions with  $P < 0.01$  are shown. Thickness is scaled with increasing significance of the  $P$  value (10).





Analysis of static networks has found that network “hubs”, i.e., genes with many interactions, modulate a variety of cellular functions and are more likely to be essential for viability (12). For the differential network, we found that the number of interactions per gene was correlated with the sensitivity to MMS of the corresponding gene deletion strain ( $r = 0.35$ ,  $P < 10^{-5}$ ) (fig. S5, A and B). Differential interaction hubs were also more likely to be essential for growth under a variety of drug treatments and stresses (fig. S5, C and D), consistent with previous observations for static hubs (13).

Further investigation showed that many differential interaction hubs were already well known to function as key components of DNA repair pathways (Fig. 2), which led us to predict that the remaining hubs might encode this role. Two such differential interaction hubs encode Slr2 and Bck1, mitogen-activated protein kinases (MAPKs) that have been implicated in the maintenance of cell wall integrity but not yet linked to DNA repair (Fig. 2). We found that Slr2 is both up-regulated and translocated to the nucleus upon MMS treatment, and it is required for appropriate regulation of ribonucleotide reductase genes in response to DNA damage (fig. S6, A to D) (14). Furthermore, both MAPKs show strong genetic interactions with DNA damage checkpoint genes (fig. S6, E and F), which suggests that they may function in a parallel signaling pathway.

Another differential interaction hub not previously linked to DNA repair was centromere binding factor 1 (*CBF1*) (Fig. 2), a sequence-specific transcription factor and component of the inner kinetochore (15). *CBF1* gained strong genetic interactions upon MMS treatment (Fig. 3A), which suggested an additional role in DNA repair. We found that Cbf1 is required for appropriate activation of cell cycle checkpoints (Fig. 3B) and that *CBF1* overexpression interferes with induction of the damage-dependent histone modification  $\gamma$ H2AX (Fig. 3C) and leads to cell cycle arrest in  $G_1$  (Fig. 3D). Furthermore, MMS treatment causes the *CBF1* profile of genetic interaction scores (across all genes on the E-MAP) to become correlated with the profiles of *TEL1* kinase and *PPH3* phosphatase, which encode key proteins regulating the DNA damage checkpoint (Fig. 3E) (16). Mass spectrometry-based phosphoproteomics showed that Cbf1 is hyperphosphorylated at a conserved serine-glutamine motif (SQ145-146) in *pph3Δ* cells (Fig. 3F and table S3). As the checkpoint kinases Mec1 and Tel1 have been shown to target Cbf1 at the same SQ site (17), it is likely that Pph3 is the protein phosphatase that counteracts the effect of this phosphorylation.

As another means of mapping DNA repair pathways, we identified genes with genetic interaction profiles that were conditionally disrupted by MMS, suggesting a shift in gene function. We observed that most genes had high correlation between their genetic interaction profiles mea-

sured in the presence or absence of MMS (high “genetic autocorrelation”) (Fig. 3G). However, several genetic interaction profiles were markedly disrupted in MMS (low autocorrelation), including those of *RAD52*, a critical factor in homologous recombination-mediated DNA repair (18), and *HTZ1*, encoding the histone variant H2A.Z, whose role in DNA repair is less well understood (19, 20). In untreated conditions, the *HTZ1* profile correlated with members of the SWR complex (*SWR1*, *SWC5*, *VPS71*, and *VPS72*) (11), responsible for incorporating Htz1 into chromatin (21). This correlation was lost in MMS (Fig. 3H), which suggested a functional disassociation between Htz1 and the SWR-C. Conversely, *HTZ1* became correlated with the DNA-damage checkpoint kinase *MEC1* upon MMS treatment (Fig. 3, H and I), and a *mec1Δhtz1Δ* strain showed synthetic sensitivity to MMS (fig. S7), suggesting a damage-dependent functional link between the two proteins. Htz1 is acetylated on its amino terminus by histone acetyltransferase NuA4 (22) and deacetylated by histone deacetylase Hda1 (19). We found that the degree of Htz1 acetylation at multiple lysine residues was strongly reduced in response to MMS (Fig. 3J). This effect was dependent on both Hda1 (Fig. 3J) and Mec1 (Fig. 3K), which suggested that the regulation of Htz1 acetylation contributes to the DNA damage response.

We next investigated the association between differential genetic interactions and known yeast pathways and protein complexes (i.e., modules) (table S1). In contrast to static genetic interactions, which are enriched within modules, we found that differential genetic interactions are not (fig. S8A). Rather, differential genetic interactions are much more likely to occur among pairs of genes connecting two different modules than among pairs of genes within the same module (fig. S8B). These findings were corroborated by an alternative analysis in which modules were defined through hierarchical clustering of the treated and untreated genetic interaction data. Genes that clustered into the same module in both conditions were much more likely to physically interact than genes that coclustered in one condition only (fig. S8C). These results suggest that known protein complexes tend to be stable across conditions—it is the genetic interactions between these modules that are reprogrammed in response to perturbation (Fig. 4A).

On the basis of these findings, we constructed a global map of gene modules and their dynamic genetic interactions in response to DNA damage. Using an established method (23), we defined modules as dense clusters of physical and static genetic interactions. Module-module interactions were characterized by heavy enrichment for many differential genetic interactions across the two modules (table S4) (10). The resulting map of 56 multigenic modules and 66 module-module interactions (Fig. 4, B and C) provides a global resource of pathways and complexes that are reconfigured in response to DNA

damage-induced stress, many of which have not been previously linked to DNA repair.

Large-scale genetic interaction networks have proved extremely powerful for mapping the pathways that regulate essential cell functions. In this study, we have shown that differential genetic networks are comparable in size to static networks, yet access a very different set of interactions governing a dynamic cellular response. Given that most gene functions arise in response to changing conditions, the differential network revealed here offers a glimpse into a much larger universe of genetic interactions that are condition-, cell type-, or tissue-specific.

## References and Notes

1. C. Boone, H. Bussey, B. J. Andrews, *Nat. Rev. Genet.* **8**, 437 (2007).
2. M. Costanzo *et al.*, *Science* **327**, 425 (2010).
3. X. Pan *et al.*, *Cell* **124**, 1069 (2006).
4. B. Lehner, C. Crombie, J. Tischler, A. Fortunato, A. G. Fraser, *Nat. Genet.* **38**, 896 (2006).
5. A. Roguev *et al.*, *Science* **322**, 405 (2008).
6. J. C. Game, R. K. Mortimer, *Mutat. Res.* **24**, 281 (1974).
7. R. P. St Onge *et al.*, *Nat. Genet.* **39**, 199 (2007).
8. M. Schuldiner, S. R. Collins, J. S. Weissman, N. J. Krogan, *Methods* **40**, 344 (2006).
9. S. R. Collins, M. Schuldiner, N. J. Krogan, J. S. Weissman, *Genome Biol.* **7**, R63 (2006).
10. Materials and methods are available as supporting material on Science Online.
11. S. R. Collins *et al.*, *Nature* **446**, 806 (2007).
12. H. Jeong, S. P. Mason, A. L. Barabási, Z. N. Oltvai, *Nature* **411**, 41 (2001).
13. A. Jaimovich, R. Rinott, M. Schuldiner, H. Margalit, N. Friedman, *Bioinformatics* **26**, i228 (2010).
14. S. J. Elledge, R. W. Davis, *Mol. Cell. Biol.* **7**, 2783 (1987).
15. M. Cai, R. W. Davis, *Cell* **61**, 437 (1990).
16. J. Heideker, E. T. Lis, F. E. Romesberg, *Cell Cycle* **6**, 3058 (2007).
17. M. B. Smolka, C. P. Albuquerque, S. H. Chen, H. Zhou, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 10364 (2007).
18. F. Pâques, J. E. Haber, *Microbiol. Mol. Biol. Rev.* **63**, 349 (1999).
19. Y. Y. Lin *et al.*, *Genes Dev.* **22**, 2062 (2008).
20. M. Papamichos-Chronakis, J. E. Krebs, C. L. Peterson, *Genes Dev.* **20**, 2437 (2006).
21. N. J. Krogan *et al.*, *Mol. Cell* **12**, 1565 (2003).
22. M. C. Keogh *et al.*, *Genes Dev.* **20**, 660 (2006).
23. S. Bandyopadhyay, R. Kelley, N. J. Krogan, T. Ideker, *PLOS Comput. Biol.* **4**, e1000065 (2008).
24. T. J. Begley, A. S. Rosenbach, T. Ideker, L. D. Samson, *Mol. Cancer Res.* **1**, 103 (2002).
25. The authors thank S. Collins, H. Hombauer, A. Desai, S. Gasser, X. Shen, and S. Choi for helpful discussions and strains. This work was funded by NIH grants R01-ES14811 and R01-GM084279. Additionally, W.-K.H. and M.-K.S. were funded by the 21C Frontier Functional Proteomics Project (FPR08A1-060), R.A. and B.B. were supported by SystemsX.ch and the Swiss National Science Foundation, and M.-C.K. was supported by the NCI (P30CA013330). N.J.K. is a Keck Young Investigator Fellow and a Searle Fellow. T.J. is a David and Lucille Packard Fellow. R.D.K. is a paid consultant to On-Q-ity.

## Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6009/1385/DC1  
Materials and Methods  
Figs. S1 to S8  
Tables S1 to S6  
References

26 July 2010; accepted 22 October 2010  
10.1126/science.1195618

# BID, BIM, and PUMA Are Essential for Activation of the BAX- and BAK-Dependent Cell Death Program

Decheng Ren,<sup>1\*</sup> Ho-Chou Tu,<sup>1\*</sup> Hyungjin Kim,<sup>1</sup> Gary X. Wang,<sup>1</sup> Gregory R. Bean,<sup>1</sup> Osamu Takeuchi,<sup>2</sup> John R. Jeffers,<sup>3</sup> Gerard P. Zambetti,<sup>3</sup> James J.-D. Hsieh,<sup>1</sup> Emily H.-Y. Cheng<sup>1,4,†‡</sup>

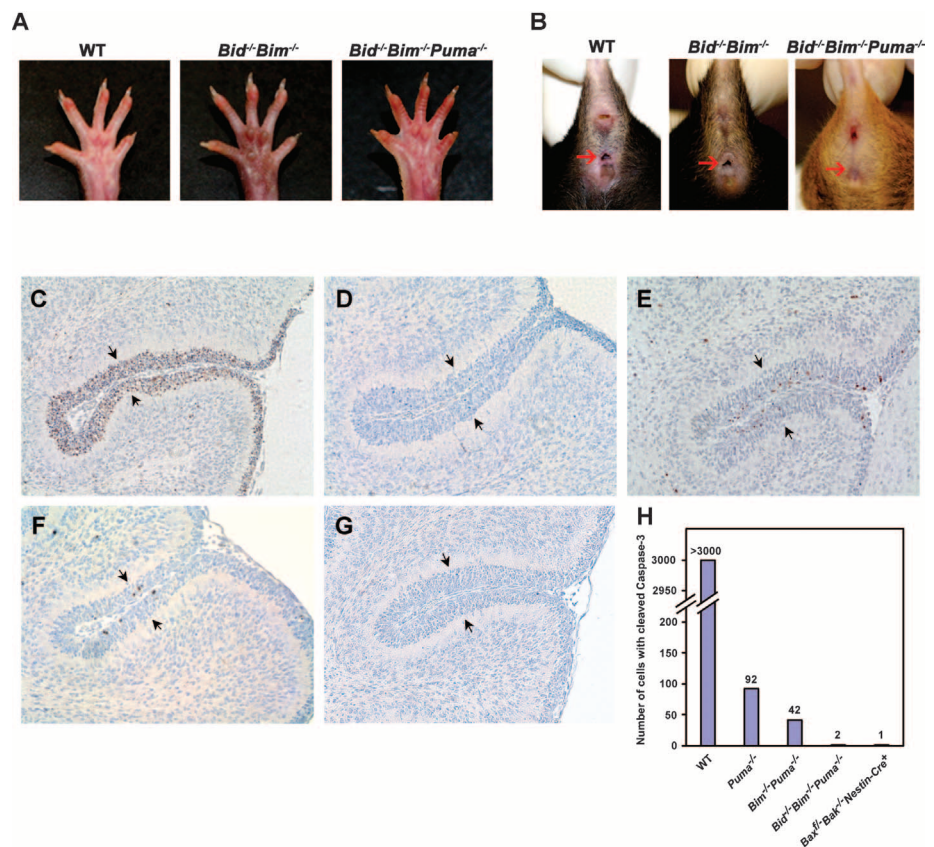
Although the proteins BAX and BAK are required for initiation of apoptosis at the mitochondria, how BAX and BAK are activated remains unsettled. We provide *in vivo* evidence demonstrating an essential role of the proteins BID, BIM, and PUMA in activating BAX and BAK. *Bid*, *Bim*, and *Puma* triple-knockout mice showed the same developmental defects that are associated with deficiency of *Bax* and *Bak*, including persistent interdigital webs and imperforate vaginas. Genetic deletion of *Bid*, *Bim*, and *Puma* prevented the homo-oligomerization of BAX and BAK, and thereby cytochrome *c*-mediated activation of caspases in response to diverse death signals in neurons and T lymphocytes, despite the presence of other BH3-only molecules. Thus, many forms of apoptosis require direct activation of BAX and BAK at the mitochondria by a member of the BID, BIM, or PUMA family of proteins.

**M**itochondria have key roles in mammalian apoptosis, a highly regulated genetic program of cell suicide (1–3). Multiple apoptotic signals release cytochrome *c* from the mitochondria to activate the Apaf-1 protein, which activates caspases. The BCL-2 family of proteins integrates developmental and environmental cues to dictate the survival or death decision of cells by regulating the integrity of the mitochondrial outer membrane (MOM) (1, 4, 5). The multidomain proapoptotic proteins BAX and BAK mediate permeabilization of the MOM,

whereas antiapoptotic BCL-2, BCL-X<sub>L</sub>, and MCL-1 proteins prevent cytochrome *c* efflux triggered by apoptotic stimuli. The third BCL-2 subfamily of proteins, the BH3-only molecules (BH3s) (that is, family members with only one BCL-2-homology domain), constitutes the largest BCL-2 subfamily with more than 10 members that promote apoptosis by either activating BAX and BAK directly or inactivating BCL-2, BCL-X<sub>L</sub>, or MCL-1 (6–12). When apoptosis is initiated, BAK and BAX undergo conformational changes to form homo-oligomers that mediate cytochrome

*c* efflux (6–9). Although BAX and BAK control the mitochondrial gateway to apoptosis, how BAX and BAK are activated, whether BAX and BAK are activated directly or indirectly by BH3s, and the identity of the core repertoire of activators of BAX and BAK in various tissues remain unsettled. Two non-mutually exclusive models have been proposed (5, 13). The direct activation model states that the “activator” subgroup of BH3s, including truncated BID (tBID) and BIM, can directly induce the conformational changes of BAX and BAK (6–12, 14–16). The indirect model proposes that activation of BAX and BAK occurs by default as long as all the antiapoptotic BCL-2 proteins are neutralized by BH3s, based on the observation that BAX- or BAK-dependent apoptosis proceeds in the absence of BID and BIM (17). However, PUMA appears also to function as a direct activator of BAX and BAK. *In vitro* translated PUMA protein, but not the BH3 domain

**Fig. 1.** Triple deficiency of *Bid*, *Bim*, and *Puma* phenocopies double deficiency of *Bax* and *Bak*. (A) *Bid*<sup>−/−</sup>*Bim*<sup>−/−</sup>*Puma*<sup>−/−</sup> TKO mice display persistence of interdigital webs. Ventral views of paws from WT, *Bid*<sup>−/−</sup>*Bim*<sup>−/−</sup>, and *Bid*<sup>−/−</sup>*Bim*<sup>−/−</sup>*Puma*<sup>−/−</sup> mice. (B) *Bid*<sup>−/−</sup>*Bim*<sup>−/−</sup>*Puma*<sup>−/−</sup> TKO mice fail to develop external vaginal introituses. Photographs of vaginal openings from WT, *Bid*<sup>−/−</sup>*Bim*<sup>−/−</sup>, and *Bid*<sup>−/−</sup>*Bim*<sup>−/−</sup>*Puma*<sup>−/−</sup> mice. Arrows point to external vaginal region. (C to G) Immunohistochemistry for cleaved caspase-3 from cerebella of postnatal day 5 (P5) mice of the indicated genotypes that were irradiated with 14 Gy  $\gamma$ -irradiation. (C) WT, 18 hours after IR. (D) *Bax*<sup>−/−</sup>*Bak*<sup>−/−</sup>*Nestin-Cre*<sup>+</sup>, 30 hours after IR. (E) *Puma*<sup>−/−</sup>, 30 hours after IR. (F) *Bim*<sup>−/−</sup>*Puma*<sup>−/−</sup>, 30 hours after IR. (G) *Bid*<sup>−/−</sup>*Bim*<sup>−/−</sup>*Puma*<sup>−/−</sup>, 30 hours after IR. Arrows denote the external granular layer of cerebellum. Data shown are representative images of two to three independent experiments. (H) Analyses of a whole sagittal section of cerebellum at the same level from experiments shown in (C) to (G) summarize the numbers of neurons at the external granular layer that were stained positive for cleaved caspase-3. Data shown are the average of two independent experiments (*n* = 2).



<sup>1</sup>Molecular Oncology, Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA. <sup>2</sup>Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Osaka 565-0871, Japan. <sup>3</sup>St. Jude Children's Research Hospital, Memphis, TN 38105, USA. <sup>4</sup>Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO 63110, USA.

\*These authors contributed equally to this work.

†Present address: Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA.

‡To whom correspondence should be addressed. E-mail: chenge1@mskcc.org

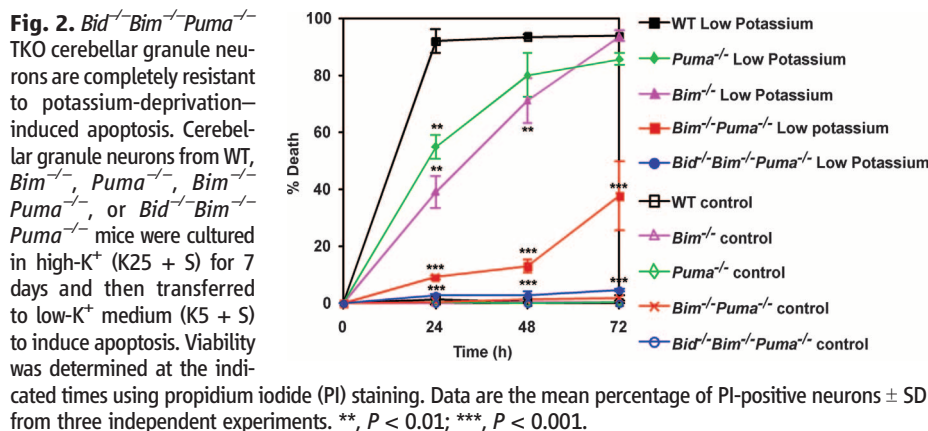


peptide of PUMA, can directly activate BAX- and BAK-dependent permeabilization of the MOM (11, 12, 18–20). The transmembrane domain was important for PUMA to induce cytochrome c efflux (fig. S1), which may explain why BH3 peptides are less active (11, 19). Thus, we studied *Bid*<sup>-/-</sup>*Bim*<sup>-/-</sup>*Puma*<sup>-/-</sup> triple-knockout (TKO) mice to clarify how BAX and BAK are activated and whether BID, BIM, and PUMA represent the core repertoire of activators of BAX and BAK that function downstream of other BH3s.

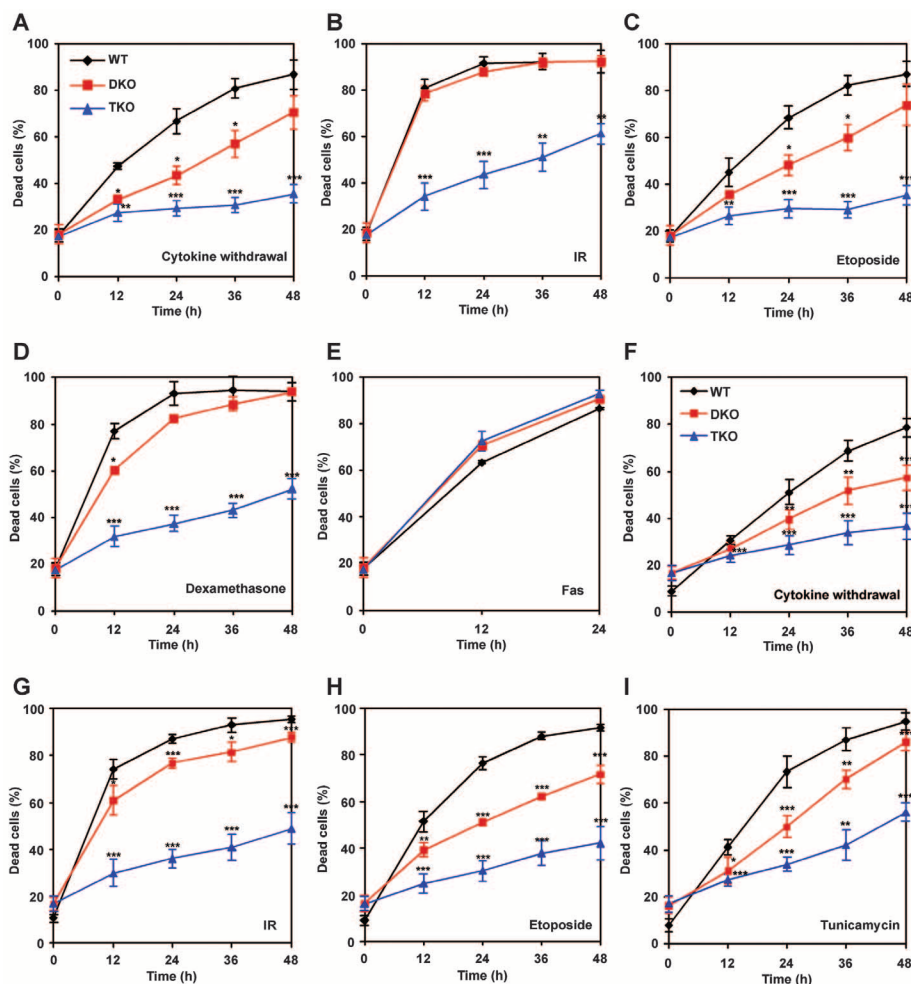
Although the embryonic lethality caused by triple deficiency of *Bid*, *Bim*, and *Puma* appeared to be less severe than that in mice lacking *Bax* and *Bak* (table S1), the viable *Bid*<sup>-/-</sup>*Bim*<sup>-/-</sup>*Puma*<sup>-/-</sup> mice displayed developmental defects very similar to those of *Bax*<sup>-/-</sup>*Bak*<sup>-/-</sup> animals, including persistent interdigital webs of skin on their feet and imperforate vaginas (21) (Fig. 1, A and B, and table S2). To determine whether deficiency of *Bid*, *Bim*, and *Puma* completely blocks the intrinsic apoptotic death as deficiency of *Bax* and

*Bak* does, we examined ionizing radiation (IR)-induced apoptosis in cerebellar granule neurons (CGN). Because most of the *Bax*<sup>-/-</sup>*Bak*<sup>-/-</sup> mice die in embryogenesis, we used the Cre-LoxP conditional knockout strategy to delete *Bax* in neurons through a Nestin-Cre construct that is expressed in neuronal and glial cell precursors (22). No activated caspase-3 was detected in *Bax*<sup>f/f</sup>*Bak*<sup>f/f</sup>*Nestin-Cre*<sup>+</sup> neurons (Fig. 1D). Puma-deficient neurons were less sensitive to IR than *Bax*-deficient neurons (Fig. 1E and fig. S2). Although deficiency of *Puma* greatly reduced the activation of caspase-3, deletion of *Bid*, *Bim*, and *Puma* was required to completely block caspase-mediated apoptosis (Fig. 1, E to H). Similar findings were also observed in IR-induced apoptosis in dentate gyrus. BAD and BMF proteins were detected in CGN (fig. S3), suggesting that BAD and BMF were unable to activate BAX- or BAK-dependent apoptosis in the absence of *Bid*, *Bim*, and *Puma*. These data indicate that activation of BAX and BAK in response to IR is fully dependent on BID, BIM, and PUMA.

To exclude the possibility that BID, BIM, and PUMA are required to activate BAX or BAK only in response to genotoxic stress, we investigated potassium-deprivation-induced apoptosis of cultured CGN. CGN from early postnatal mice



**Fig. 3.** *Bid*<sup>-/-</sup>*Bim*<sup>-/-</sup>*Puma*<sup>-/-</sup> TKO T cells are resistant to diverse apoptotic stimuli. (A to E) CD4<sup>+</sup> T cells purified from the spleens of WT (*n* = 3), *Bid*<sup>-/-</sup>*Bim*<sup>-/-</sup> (*n* = 3), or *Bid*<sup>-/-</sup>*Bim*<sup>-/-</sup>*Puma*<sup>-/-</sup> (*n* = 3) mice at 8 to 10 weeks of age were cultured under the following conditions: in the absence of cytokine (A), after exposure to 2.5 Gy  $\gamma$ -irradiation (B), in the presence of etoposide (C), in the presence of dexamethasone (D), or in the presence of agonistic antibody to Fas (E). Cell death was quantified by annexin-V staining at the indicated times. (F to I) Thymocytes from WT (*n* = 3), *Bid*<sup>-/-</sup>*Bim*<sup>-/-</sup> (*n* = 3), or *Bid*<sup>-/-</sup>*Bim*<sup>-/-</sup>*Puma*<sup>-/-</sup> (*n* = 3) mice at 6 to 8 weeks of age were cultured under the following conditions: in the absence of cytokine (F), after exposure to 2.5 Gy  $\gamma$ -irradiation (G), in the presence of etoposide (H), or in the presence of tunicamycin (I). Cell death was quantified by annexin-V staining at the indicated times. Data are the mean percentage of annexin-V-positive cells ± SD from three independent experiments. \*, *P* < 0.05; \*\*, *P* < 0.01; \*\*\*, *P* < 0.001.



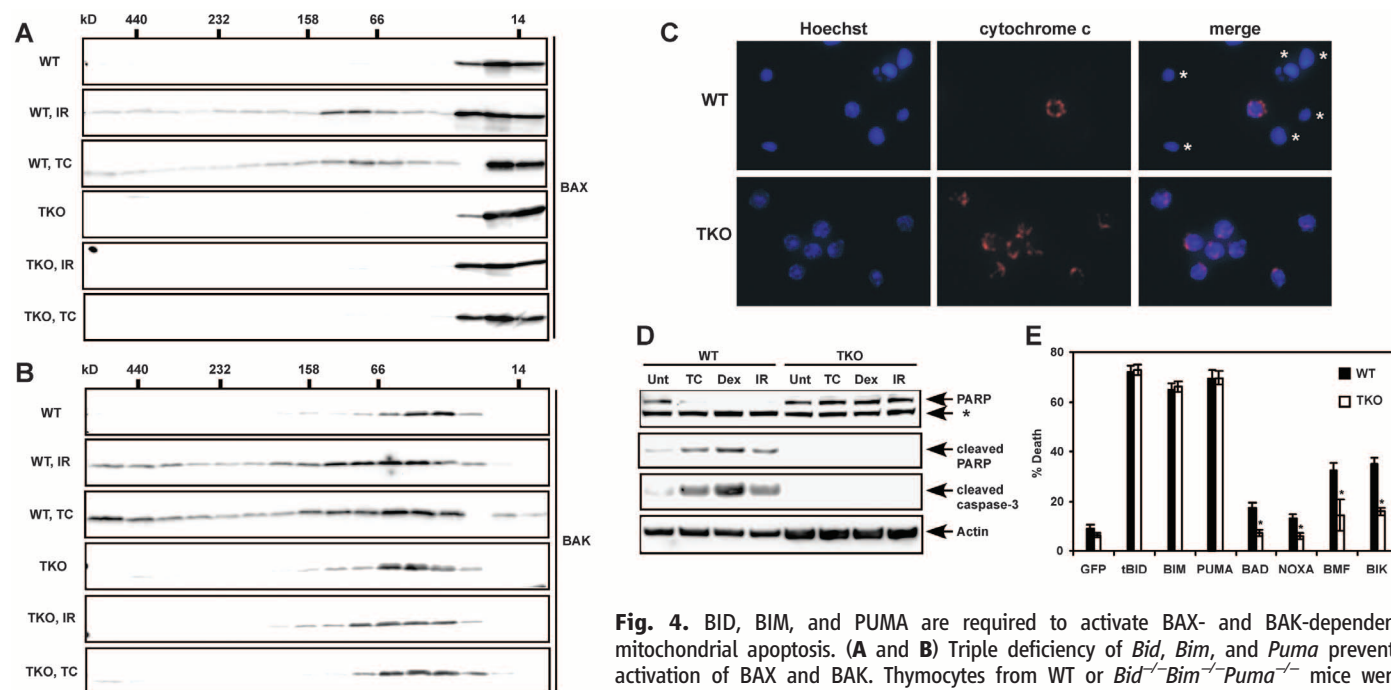
can be cultured in medium containing a high concentration of potassium (25 mM) that provides depolarization-mediated survival. Deprivation of potassium (5 mM) induces BAX-dependent apoptosis that requires de novo protein synthesis (23). Consistent with the previous reports demonstrating the accumulation of BIM and PUMA proteins in potassium-deprived CGN (24, 25), deficiency of either *Bim* or *Puma* provided transient protection, whereas deficiency of both *Bim* and *Puma* conferred long-term protection against potassium-deprivation-induced apoptosis (Fig. 2 and figs. S4 and S5). Deletion of *Bid*, *Bim*, and *Puma* completely blocked apoptosis up to 3 days (Fig. 2 and figs. S4 and S5). These data indicate that BAX is not activated in the absence of *Bid*, *Bim*, and *Puma*, even though BAD and BMF are present (fig. S3) and the abundance of HRK (also called DP5) is increased in these neurons (26).

One of the major phenotypes of *Bax*<sup>-/-</sup> *Bak*<sup>-/-</sup> mice is the accumulation of lymphoid and myeloid cells as a consequence of defective apoptosis (21). *Bid*<sup>-/-</sup> *Bim*<sup>-/-</sup> *Puma*<sup>-/-</sup> animals also exhibited enlarged thymi, splenomegaly, and lymphadenopathy (fig. S6). The role of PUMA as a direct activator of BAX and BAK is debated in part because of reports showing that knockdown of *Puma* does not provide further inhibition of apoptosis in the absence of *Bid* and *Bim* (17) and because exogenous

PUMA fails to induce BAX- and BAK-dependent mitochondrial permeabilization in the absence of *Bid* and *Bim* (27). To address whether endogenous PUMA can activate BAX- and BAK-dependent mitochondrial apoptosis independently of BID and BIM, we compared the apoptotic phenotypes of *Bid*<sup>-/-</sup> *Bim*<sup>-/-</sup> double-knockout (DKO) mice to *Bid*<sup>-/-</sup> *Bim*<sup>-/-</sup> *Puma*<sup>-/-</sup> TKO mice. The TKO mice had more pronounced accumulation of hematopoietic cells in thymus, spleen, lymph nodes, and blood than DKO mice (figs. S6 to S8). Moreover, *Bid*<sup>-/-</sup> *Bim*<sup>-/-</sup> *Puma*<sup>-/-</sup> TKO T cells were more resistant than *Bid*<sup>-/-</sup> *Bim*<sup>-/-</sup> DKO cells to diverse intrinsic apoptotic signals, including cytokine deprivation, DNA damage (IR or etoposide), glucocorticoids (dexamethasone), or endoplasmic reticulum (ER) stress (tunicamycin) (Fig. 3 and fig. S9). *Puma*-deficient cells were less resistant to apoptosis than TKO cells (fig. S10). Indeed, deficiency of *Bid*, *Bim*, and *Puma* appears to provide more protection against intrinsic apoptotic death than deficiency of *Apaf-1* or *Caspase-9* (28). By analogy to *Bax*<sup>-/-</sup> *Bak*<sup>-/-</sup> T cells (21), *Bid*<sup>-/-</sup> *Bim*<sup>-/-</sup> *Puma*<sup>-/-</sup> T cells were still sensitive to Fas-induced apoptosis because the BCL-2 family does not regulate extrinsic apoptotic death in T cells (Fig. 3E). The proapoptotic activity of BID is activated upon its cleavage by proteases such as caspase-8 or -2 (1, 4, 5).

Cleavage of BID was detected in thymocytes in response to DNA damage or ER stress (fig. S11).

To investigate whether activation of BAX and BAK was blocked in the absence of *Bid*, *Bim*, and *Puma*, gel filtration was performed to assess homo-oligomerization of BAX or BAK. In both wild-type (WT) and *Bid*<sup>-/-</sup> *Bim*<sup>-/-</sup> *Puma*<sup>-/-</sup> TKO cells, BAX was eluted as a 20-kD monomer from untreated cells (Fig. 4A). After IR or treatment of cells with tunicamycin, BAX formed higher-ordered oligomers in WT but not TKO cells (Fig. 4A). Similarly, higher-ordered oligomers of BAK were only detected in WT cells in response to genotoxic or ER stress (Fig. 4B). Consistent with the lack of homo-oligomerization of BAX or BAK detected in TKO cells, cytochrome c translocation and caspase activation were not observed in these cells when exposed to diverse intrinsic apoptotic signals (Fig. 4, C and D, and figs. S12 and S13). Although the majority of WT cells lost cytochrome c immunostaining within 20 hours after IR, in TKO cells, cytochrome c remained in the mitochondria, exhibiting a punctate staining pattern (Fig. 4C). Caspase activation determined by the cleavage of poly (ADP-ribose) polymerase (PARP) and caspase-3 was not observed in TKO cells treated with IR, dexamethasone, or tunicamycin (Fig. 4D). Notably, tBID, BIM, and PUMA were potent death agonists in both WT



**Fig. 4.** BID, BIM, and PUMA are required to activate BAX- and BAK-dependent mitochondrial apoptosis. (A and B) Triple deficiency of *Bid*, *Bim*, and *Puma* prevents activation of BAX and BAK. Thymocytes from WT or *Bid*<sup>-/-</sup> *Bim*<sup>-/-</sup> *Puma*<sup>-/-</sup> mice were untreated, irradiated with 5 Gy  $\gamma$ -irradiation (IR), or treated with tunicamycin (TC). Protein lysates were harvested at 7 hours after IR or 20 hours after TC treatment and subjected to Superdex 200 (HR10/30) gel-filtration chromatography. Fractions were analyzed by Western blot using antibodies to BAX (A) or BAK (B). (C) Triple deficiency of *Bid*, *Bim*, and *Puma* prevents the translocation of cytochrome c. Fluorescence microscopy of WT or *Bid*<sup>-/-</sup> *Bim*<sup>-/-</sup> *Puma*<sup>-/-</sup> thymocytes 20 hours after exposure to 2.5 Gy  $\gamma$ -irradiation. Red represents cytochrome c immunostaining, and blue is Hoechst staining. White asterisks indicate apoptotic cells that have lost cytochrome c. (D) Triple deficiency of *Bid*, *Bim*, and *Puma* prevents the activation of caspases. Thymocytes from WT or *Bid*<sup>-/-</sup> *Bim*<sup>-/-</sup> *Puma*<sup>-/-</sup> mice were untreated, treated with TC or dexamethasone (Dex), or irradiated with 5 Gy  $\gamma$ -irradiation (IR). After 12 hours, protein lysates were harvested and assessed by Western blot using antibodies specific for PARP, cleaved PARP, cleaved caspase-3, or actin. Asterisk denotes a cross-reactive protein. (E) Primary mouse embryonic fibroblasts isolated from WT or *Bid*<sup>-/-</sup> *Bim*<sup>-/-</sup> *Puma*<sup>-/-</sup> TKO mice were infected with retroviruses expressing the indicated genes. Cell death was quantified by annexin-V staining at 24 hours. Data are the mean percentage of annexin-V-positive cells  $\pm$  SD from three independent experiments. \*,  $P < 0.05$ .



and *Bid*<sup>-/-</sup> *Bim*<sup>-/-</sup> *Puma*<sup>-/-</sup> TKO cells (Fig. 4E and fig. S14).

The lack of strong and stable interaction between BH3s and BAX/BAK was thought to support the indirect activation model for BAX and BAK. However, dynamic interactions occur between BAX- and BAK-dependent BH3s, including tBID, BIM, and PUMA (18), which helps explain the previous difficulty in detecting these interactions. Here, we demonstrate an essential role of BID, BIM, and PUMA in activating BAX and BAK and for some apoptotic events during development. Our genetic study indicates that BAD is unable to induce apoptosis in the absence of BID, BIM, and PUMA, which is further supported by the observation that the BAD mimetic, ABT-737 (29), failed to kill *Bid*<sup>-/-</sup> *Bim*<sup>-/-</sup> *Puma*<sup>-/-</sup> TKO cells (fig. S15). These data suggest that the profound block of apoptosis conferred by the triple deficiency of *Bid*, *Bim*, and *Puma* is not simply caused by altering the ratio between antiapoptotic and proapoptotic BCL-2 proteins. BH3s not only induce BAX- and BAK-dependent release of cytochrome c to activate caspases but also initiate caspase-independent mitochondrial dysfunction (9). Accordingly, persistent interdigital webs and accumulation of hematopoietic cells were ob-

served in *Bax*<sup>-/-</sup> *Bak*<sup>-/-</sup> DKO (21) or *Bid*<sup>-/-</sup> *Bim*<sup>-/-</sup> *Puma*<sup>-/-</sup> TKO mice, but not in *Apaf-1*-deficient mice (28, 30). Overall, our study reveals an essential axis of activator BH3s and BAX and BAK in activating the mitochondrial death program, which offers common ground for therapeutic interventions.

#### References and Notes

1. A. Gross, J. M. McDonnell, S. J. Korsmeyer, *Genes Dev.* **13**, 1899 (1999).
2. X. Wang, *Genes Dev.* **15**, 2922 (2001).
3. D. D. Newmeyer, S. Ferguson-Miller, *Cell* **112**, 481 (2003).
4. J. C. Reed, *Cell Death Differ.* **13**, 1378 (2006).
5. R. J. Youle, A. Strasser, *Nat. Rev. Mol. Cell Biol.* **9**, 47 (2008).
6. S. Desagher et al., *J. Cell Biol.* **144**, 891 (1999).
7. M. C. Wei et al., *Genes Dev.* **14**, 2060 (2000).
8. M. C. Wei et al., *Science* **292**, 727 (2001).
9. E. H. Cheng et al., *Mol. Cell* **8**, 705 (2001).
10. A. Letai et al., *Cancer Cell* **2**, 183 (2002).
11. T. Kuwana et al., *Mol. Cell* **17**, 525 (2005).
12. H. Kim et al., *Nat. Cell Biol.* **8**, 1348 (2006).
13. H. L. Galonek, J. M. Hardwick, *Nat. Cell Biol.* **8**, 1317 (2006).
14. T. Kuwana et al., *Cell* **111**, 331 (2002).
15. J. F. Lovell et al., *Cell* **135**, 1074 (2008).
16. E. Gavathiotis et al., *Nature* **455**, 1076 (2008).
17. S. N. Willis et al., *Science* **315**, 856 (2007).
18. H. Kim et al., *Mol. Cell* **36**, 487 (2009).
19. M. Certo et al., *Cancer Cell* **9**, 351 (2006).
20. N. Y. Fu, S. K. Sukumaran, S. Y. Kerk, V. C. Yu, *Mol. Cell* **33**, 15 (2009).
21. T. Lindsten et al., *Mol. Cell* **6**, 1389 (2000).
22. F. Tronche et al., *Nat. Genet.* **23**, 99 (1999).
23. T. M. Miller et al., *J. Cell Biol.* **139**, 205 (1997).
24. G. V. Putcha et al., *J. Cell Biol.* **157**, 441 (2002).
25. C. G. Besirli, E. F. Wagner, E. M. Johnson Jr., *J. Cell Biol.* **170**, 401 (2005).
26. C. A. Harris, E. M. Johnson Jr., *J. Biol. Chem.* **276**, 37754 (2001).
27. J. E. Chipuk et al., *Proc. Natl. Acad. Sci. U.S.A.* **105**, 20327 (2008).
28. V. S. Marsden et al., *Nature* **419**, 634 (2002).
29. T. Oltersdorf et al., *Nature* **435**, 677 (2005).
30. H. Yoshida et al., *Cell* **94**, 739 (1998).
31. We apologize to all the investigators whose research could not be appropriately cited owing to space limitation. We thank T. D. Westergaard and H.-F. Chen for technical assistance. This work was supported by grants to E.H.-Y.C. from NIH (R01CA125562) and the Searle Scholars Program, and to G.P.Z. from NIH (R01GM083159 and P30CA21765).

#### Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6009/1390/DC1

Materials and Methods

Figs. S1 to S15

Tables S1 and S2

References

30 March 2010; accepted 14 October 2010

10.1126/science.1190217

# Arabidopsis Type I Metacaspases Control Cell Death

Nuria S. Coll,<sup>1</sup> Dominique Vercammen,<sup>2\*</sup> Andrea Smidler,<sup>1</sup> Charles Clover,<sup>1†</sup> Frank Van Breusegem,<sup>2</sup> Jeffery L. Dangl,<sup>1,3,4‡</sup> Petra Eppele<sup>1‡</sup>

Metacaspases are distant relatives of animal caspases found in protozoa, fungi, and plants. Limited experimental data exist defining their function(s), despite their discovery by homology modeling a decade ago. We demonstrated that two type I metacaspases, AtMC1 and AtMC2, antagonistically control programmed cell death in *Arabidopsis*. AtMC1 is a positive regulator of cell death and requires conserved caspase-like putative catalytic residues for its function. AtMC2 negatively regulates cell death. This function is independent of the putative catalytic residues. Manipulation of the *Arabidopsis* type I metacaspase regulatory module can nearly eliminate the hypersensitive cell death response (HR) activated by plant intracellular immune receptors. This does not lead to enhanced pathogen proliferation, decoupling HR from restriction of pathogen growth.

Programmed cell death is essential for plant development (1). Metacaspases in plants, fungi, and protozoa are distant homologs of

caspases in the CD cysteine protease superfamily (caspases, paracaspases, gingipains, clostripains, legumains, and separin). These evolutionarily related proteases share the caspase-hemoglobinase fold, and common catalytic-site domains (2, 3). The *Arabidopsis thaliana* genome encodes three type I and six type II metacaspases (AtMCs) (3). Both types contain a conserved putative catalytic domain and plausible sites for autocatalytic processing but differ in their N-terminal domains. Despite many reports of caspase-like activities in plants (4–9), no experimental data exist defining functions or substrates for plant type I metacaspases. Type II metacaspase functions are nearly as enigmatic: Recombinant plant type II metacaspases AtMC4 and AtMC9 can undergo in vitro autocatalytic processing (10), and the Tudor staphylococcal

nuclease, cleaved during programmed cell death in pine, is the only in vivo type II metacaspase substrate defined in plants (11). Although classic animal caspases cleave after an aspartate at the P1 position, metacaspases cleave after a basic amino acid residue such as lysine or arginine (9).

LSD1 is a negative regulator of cell death initiated by localized superoxide production occurring during the hypersensitive response (HR), a cell death that often accompanies pathogen recognition. HR sites form normally in *lsd1* (12), but the typical sharp boundary between dead and live cells subsequently breaks down and “run-away cell death” ensues throughout the leaf (12). This phenotype requires proteins that are also necessary for pathogen recognition and salicylic acid accumulation (12–16). Hence, *lsd1* is a sensitized genetic background for studying the control of oxidative stress-dependent cell death (17–20).

All three type I *Arabidopsis* metacaspases, AtMC1 (At1g02170), AtMC2 (At4g25110), and AtMC3 (At5g64240), possess a conserved, plant-specific LSD1-like zinc-finger N-terminal motif (CxxCRxxLMYxxGASxxVxCxxC) (21) (fig. S1A). The LSD1 zinc-finger domain can function in protein interactions (22). In yeast, LSD1 and AtMC1 interact via their zinc-finger domains (fig. S1, B to D). In contrast, AtMC2 interacts only very weakly with either AtMC1 or LSD1 in this assay (fig. S1, B to D). We substantiated the AtMC1-LSD1 interaction using in vivo coimmunoprecipitation in transgenic *Arabidopsis* (Fig. 1B) and in transient expression assays in *Nicotiana benthamiana* (23) (fig. S2A). The predicted N-terminal prodomain of AtMC1 is required for interaction with

<sup>1</sup>Department of Biology, 108 Coker Hall, University of North Carolina (UNC), CB 3280, Chapel Hill, NC 27599–3280, USA.

<sup>2</sup>VIB Department of Plant Systems Biology and Department of Plant Biotechnology and Genetics, Ghent University, 9052 Ghent, Belgium.

<sup>3</sup>Curriculum in Genetics and Molecular Biology and Department of Microbiology and Immunology, UNC, Chapel Hill, NC 27599, USA. <sup>4</sup>Carolina Center for Genome Sciences, UNC, Chapel Hill, NC 27599, USA.

\*Present address: Innogenetics, Technologiepark 6, 9052 Ghent, Belgium.

†Present address: Department of Anesthesiology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA.

‡To whom correspondence should be addressed. E-mail: peppele@email.unc.edu (P.E.); dangl@email.unc.edu (J.L.D.)

LSD1 (fig. S2A). AtMC2 does not coimmunoprecipitate with either LSD1 (fig. S2B) or AtMC1 (fig. S2C) under these conditions.

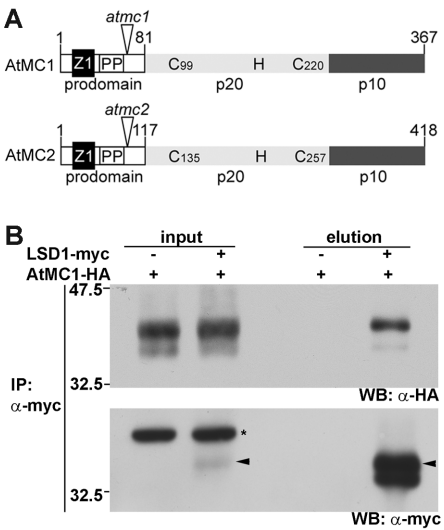
We obtained transfer DNA (T-DNA) insertional mutant alleles of *atmc1*, *atmc2*, and *lsd1* in the Col-0 accession (Fig. 1A and fig. S1B). *atmc1*, *atmc2*, and the *atmc1 atmc2* double mutant exhibited no signs of enhanced production of reactive oxygen species and had no obvious phenotypes (fig. S3). We generated *lsd1 atmc1* and *lsd1 atmc2* double mutants, and the *lsd1 atmc1 atmc2* triple mutant to determine whether *atmc1* or *atmc2* modifies the *lsd1* cell death phenotype. *lsd1* runaway cell death can be induced with benzo(1,2,3)thiadiazole-7-carboethioic acid *S*-methyl ester (BTH), a salicylic acid analog (16). *lsd1* exhibited maximum ion leakage (a cell death proxy) 96 hours after BTH treatment. The Col-0 wild type, *atmc1*, *atmc2*, and *atmc1 atmc2* did not display significant increases in ion leakage (Fig. 2A). *lsd1 atmc1* exhibited suppressed BTH-induced ion leakage (Fig. 2A, left). In contrast, *lsd1 atmc2* displayed accelerated BTH-induced ion leakage (Fig. 2A, right). Ion leakage in *lsd1 atmc1 atmc2* was similar to that in *lsd1 atmc1* (Fig. 2A, right). Consistent with these data, morphological *lsd1* phenotypes were abolished in the absence of *AtMC1*, and more pronounced in the *lsd1 atmc2* mutant (fig. S3). Hence,

AtMC1 is a positive mediator of *lsd1* runaway cell death, and AtMC2 acts genetically as a negative regulator of AtMC1.

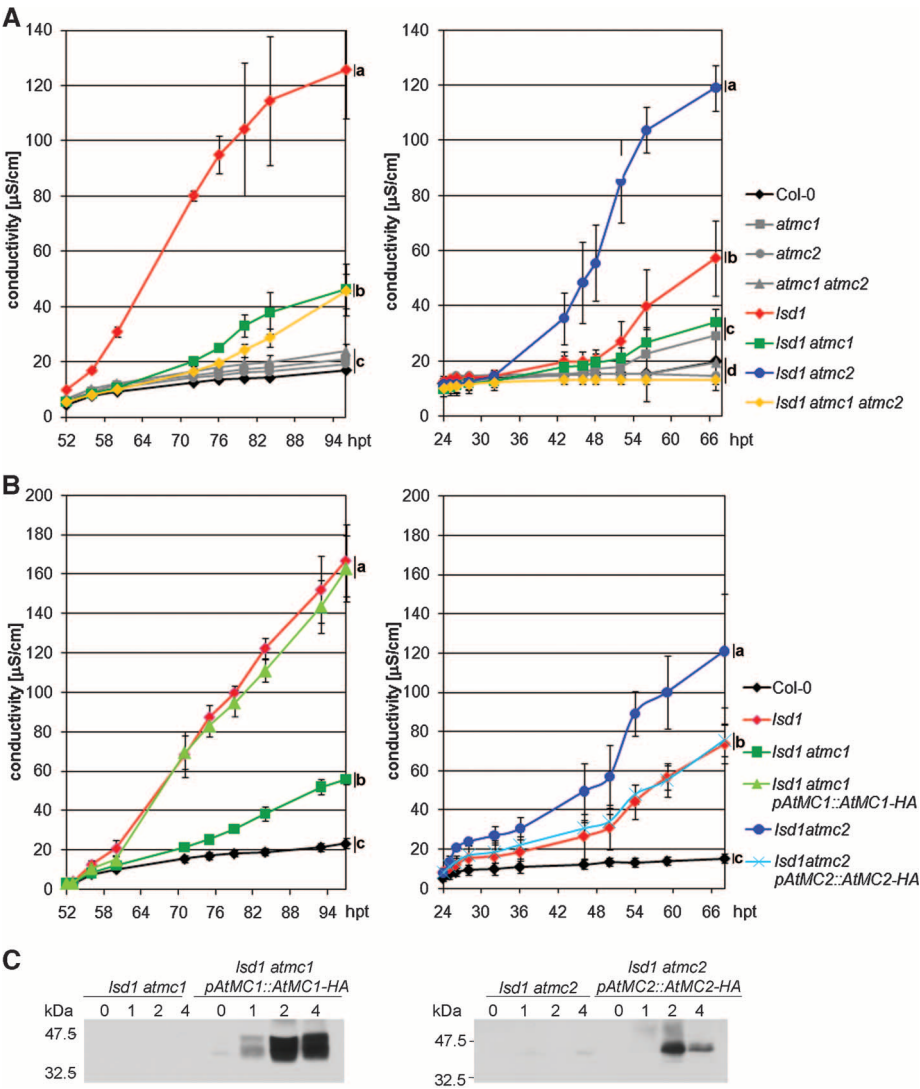
Hemagglutinin (HA)-epitope-tagged *AtMC1* and *AtMC2* constructs controlled by their native promoters (*pAtMC1::AtMC1-HA* and *pAtMC2::AtMC2-HA*) complemented the respective mutant phenotypes for BTH-induced ion leakage in *lsd1* (Fig. 2B). AtMC1-HA protein was detectable 1 day after BTH spray, before plants displayed any visible sign of cell death, and accumulated thereafter (Fig. 2C). AtMC2-HA was undetectable until 2 days after BTH treatment and accumulated to lower levels than AtMC1-HA (Fig. 2C). We observed no HA-tagged low-molecular-weight products, suggesting either a lack of processing during activation or instability of a putative C-terminal HA-tagged fragment.

We monitored *AtMC1* or *AtMC2* expression in transgenic plants carrying promoter  $\beta$ -glucuronidase (*GUS*) reporter genes (*pAtMC1::GUS* and *pAtMC2::GUS*, respectively) in either Col-0 or *lsd1*. *AtMC1* expression was confined to the leaf veins. However, 24 hours after BTH treatment, *AtMC1* was expressed in a narrow zone of several cells adjacent to cell death sites in *lsd1* (fig. S4A). Cells expressing *AtMC1* are destined to die as runaway cell death expands over time (15). *AtMC2*, in contrast, was expressed at low levels, except for a halo of nonstained cells outlining cells that are committed to die (fig. S4B).

We infected Col-0 *pAtMC1::GUS* and *pAtMC2::GUS* transgenic plants with either the obligate biotrophic oomycete *Hyaloperonospora arabidopsidis* (*Hpa*; isolate Emwa1), or the hemibiotrophic bacte-



**Fig. 1.** AtMC1 interacts with LSD1. (A) Scheme of AtMC1 and AtMC2 proteins (not to scale). Z1, LSD1-like zinc finger; PP, proline-rich region; p20 and p10, putative caspase-like catalytic domains (2); numbered H and C, putative catalytic residues (2); *atmc1* and *atmc2*, T-DNA insertion sites. (B) AtMC1 and LSD1 coimmunoprecipitate. Protein extracts from leaves of *lsd1 atmc1* [*pLSD1::LSD1-myc*]  $\times$  *lsd1 atmc1* [*Dex-AtMC1-HA*]  $F_1$  plants were immunoprecipitated with anti-myc-coupled magnetic beads. Crude extract (input) and eluate (elution) were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with anti-HA (top) or anti-myc (bottom) immunoblot. The eluate is 8 $\times$  concentrated as compared to the input. The asterisk denotes a nonspecific cross-reacting band; arrowheads are ~34 kDa, the expected apparent molecular mass of LSD1-myc. WB, Western blot.



**Fig. 2.** *AtMC1* and *AtMC2* antagonistically control *lsd1* runaway cell death. (A and B) Four-week-old homozygous transgenic plants (genotypes, right) were sprayed with 150  $\mu$ M BTH, and conductivity was measured at the time points indicated. Repeated three times in (A) and once in (B). Error bars indicate two times the standard error, calculated from four replicate measurements per genotype and data point. Letters a to d at right represent groups with significant differences [ $P < 0.05$ , Tukey's honest significant difference (HSD) test]. hpt, hours post treatment. (C) Four-week-old plants of the indicated genotypes were sprayed with 150  $\mu$ M BTH. Tissue was harvested at the indicated time points, and 50  $\mu$ g of total protein were analyzed by SDS-PAGE and anti-HA immunoblot.



ria *Pseudomonas syringae* pv. *tomato* [Pto; strain DC3000(*avrRpm1*)] to trigger HR via the specific intracellular Toll–interleukin-1 receptor (TIR) and coiled-coil (CC)–nucleotide-binding–leucine-rich repeat (NB-LRR) immune receptors RPP4 (24) and RPM1 (25), respectively. *AtMC1* was activated upon NB-LRR–based pathogen recognition and subsequently expressed in a spatially restricted manner in cells destined to undergo HR (fig. S5). In contrast, *AtMC2* was expressed in a relatively more diffuse zone around infection sites (fig. S5).

We generated transgenic plants conditionally expressing either a full-length or an N-terminal truncated version of *AtMC1* lacking the *LSD1*-like extension (*AtMC1-HA* and *AtMC1-ΔN-HA*, respectively). High levels of *AtMC1-HA* accumulation resulted in cell death in Col-0 transgenics; lower levels did not (Fig. 3A). Col-0 *AtMC1-ΔN-HA* plants accumulating relatively low levels of protein nevertheless exhibited enhanced cell death (Fig. 3A). We failed to recover transgenics expressing *AtMC1-ΔN-HA* in *lsd1* either by direct transformation or from crosses of two independent Col-0 *AtMC1-ΔN-HA*-expressing transgenic lines to *lsd1* [see methods in supporting online material (SOM)]. These results suggest that the *LSD1*-like putative prodomain of *AtMC1* negatively regulates its pro-cell death activity.

Typical caspase active sites include a histidine-cysteine catalytic dyad, also found in metacaspases (3). The predicted catalytic cysteine residue was essential for autoprocessing of the pine metacaspase mclI-Pa (26), of *Arabidopsis* *AtMC4* and *AtMC9* in bacteria (10), and of *AtMC1* and *AtMC5* in

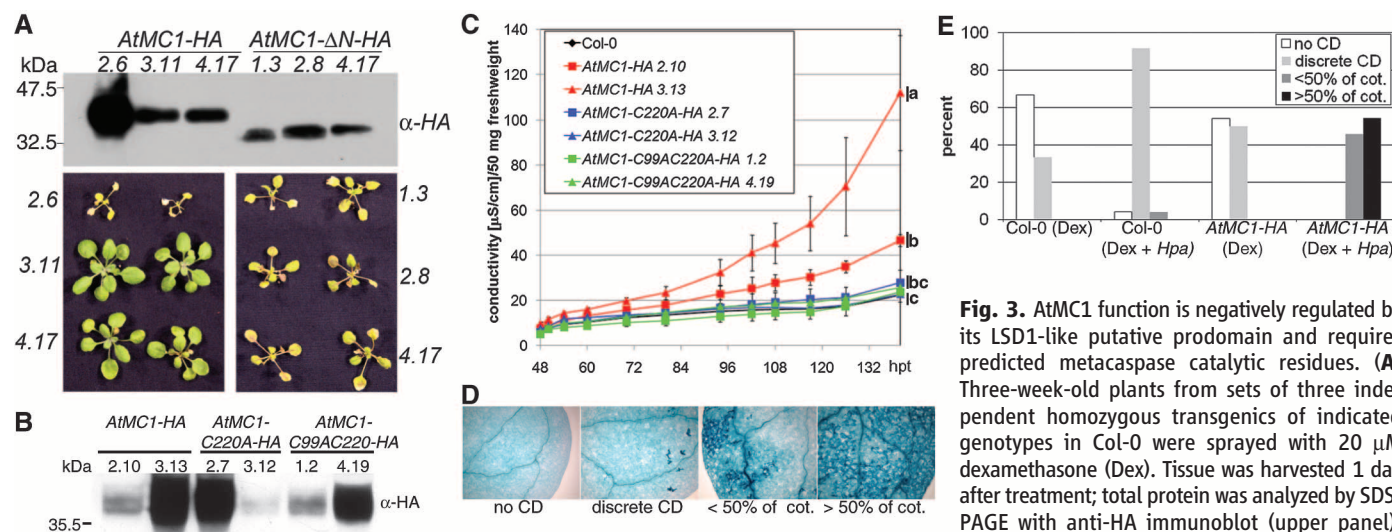
yeast (27). In *Trypanosoma brucei* metacaspase *TbMCA4*, a second, metacaspase-specific cysteine residue was catalytic, and the corresponding residue compensated for mutation of the classic cysteine residue in *AtMC9* (28, 29). We generated Col-0 transgenic lines expressing high or low levels of either a conditional *AtMC1* expression construct containing a cysteine-to-alanine mutation (*AtMC1-C220A-HA*) or a double mutation including the potentially compensatory cysteine (*AtMC1-C99A-C220A-HA*) (Fig. 3B). Conditional overexpression of *AtMC1-HA* resulted in a dose-dependent increase in ion leakage, whereas overexpression of either *AtMC1-C220A-HA* or *AtMC1-C99A-C220A-HA* did not (Fig. 3C). Hence, *AtMC1-C220* is required for function, and *AtMC1-C99* cannot compensate for its loss.

Overexpression of *AtMC1* did not induce cell death in cotyledons before infection. However, massive cell death occurred after infection with *Hpa* and activation of RPP4 (Fig. 3, D and E). Hence, *AtMC1* activation in seedlings, in contrast to adult plants (Fig. 3A), requires an additional signal, which is consistent with the fact that *lsd1* runaway cell death also does not occur in cotyledons.

We generated similar transgenics in *lsd1* and Col-0 that conditionally expressed full-length *AtMC2* (*AtMC2-HA*), an analogous N-terminal truncated version of *AtMC2* (*AtMC2-ΔN-HA*) and either *AtMC2* or *AtMC2-ΔN* with cysteine-to-alanine mutations in the predicted catalytic (C256) and potentially compensatory (C135) cysteine residues (*AtMC2-C135A-C256A-HA* and *AtMC2-ΔN-C135A-C256A-HA*). BTH-induced *lsd1* runaway cell death was suppressed in transgenic lines

expressing full-length *AtMC2-HA* (fig. S6, A and B). Cell death suppression was nearly complete in *AtMC2-ΔN-HA*-expressing lines (Fig. 4A and figs. S6C and S7). Surprisingly, the predicted catalytic cysteines were not required for cell death suppression by either *AtMC2* or *AtMC2-ΔN-HA* (Fig. 4A and fig. S6B).

To assess whether *AtMC2* suppressed NB-LRR–mediated HR, we infected Col-0, *atmc1*, *atmc2*, *atmc1 atmc2*, and transgenic plants expressing *AtMC2-ΔN-HA* or *AtMC2-ΔN-C135A-C256A-HA* with *Pto* DC3000(*avrRpm1*). We observed enhancement of RPM1-mediated HR in *atmc2* (Fig. 4B). Conversely, we observed suppression of RPM1-mediated HR in *atmc1* and in *atmc1 atmc2* at low bacterial inocula resembling natural infections (Fig. 4B), but not at artificially high doses. Conditional expression of either *AtMC2-ΔN* (Fig. 4C and fig. S6F) or full-length *AtMC2* (fig. S6, D and E) suppressed RPM1-mediated HR, phenocopying *atmc1*. The suppression of HR did not result in increased susceptibility to *Pto* DC3000(*avrRpm1*) (Fig. 4, D and E). RPP4-mediated HR was also suppressed by conditional expression of *AtMC2-ΔN* and in *atmc1* (Fig. 4F). Thus, *AtMC2* inhibits at least RPM1- and RPP4-mediated HR, via direct or indirect regulation of *AtMC1*. The putative catalytic sites of *AtMC2* are not required for HR suppression (Fig. 4, B, C, and F, and fig. S6E). *atmc2* did not exhibit increased RPP4-mediated HR (30). Although *Hpa* Emwa1 is an obligate biotrophic oomycete, *AtMC2-ΔN*-mediated suppression of HR did not abolish RPP4-dependent pathogen growth restriction (Fig. 4F). These surprising observations are consistent with pre-

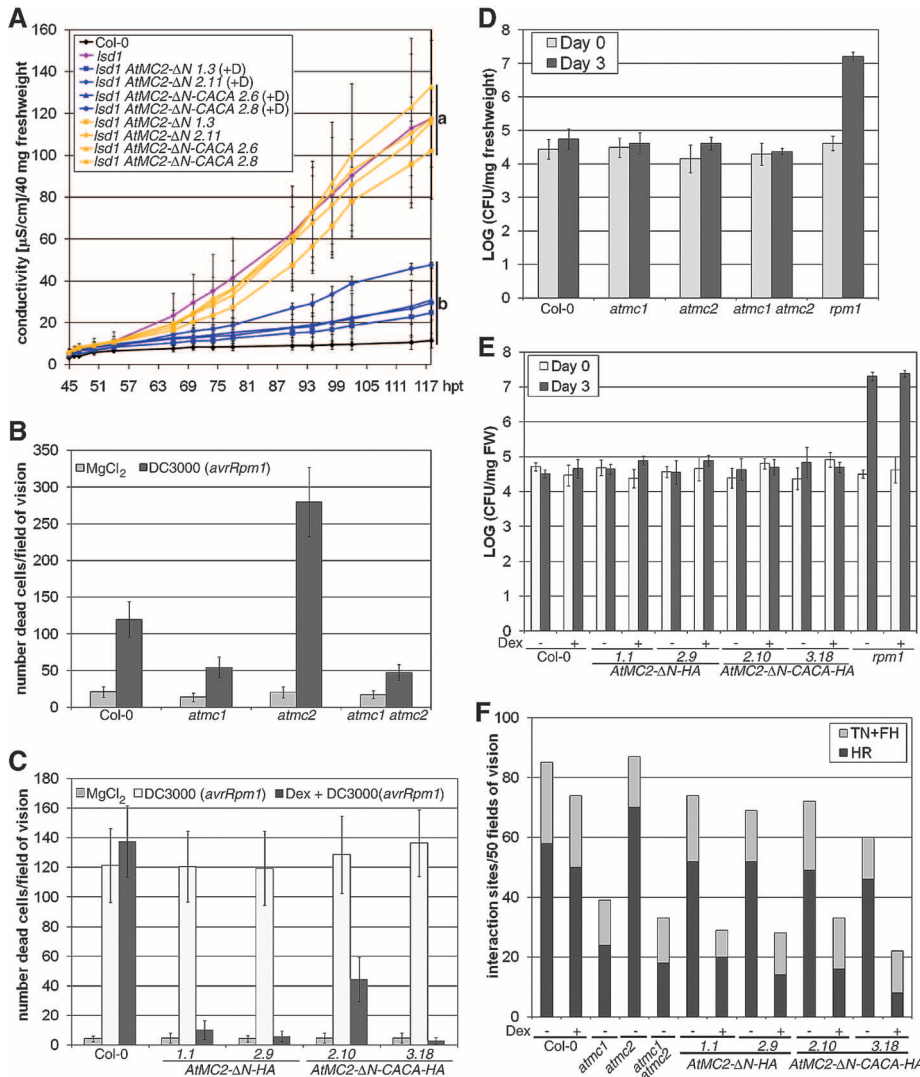


treatment are shown (lower panel). (B) As in (A), but using two independent transgenics of the genotypes listed above the blot. (C) *AtMC1*-mediated ion leakage requires the putative catalytic cysteine residue C220. Plants treated as described in (A) were harvested at 46 hpt and processed as described in the SOM. Error bars represent two times the standard error. Letters a to c represent experimental groups with significant differences ( $P < 0.05$ , Tukey's HSD test). The experiment was repeated twice. (D and E) *AtMC1* function in cotyledons requires additional signals. Ten-day-old seedlings were pre-treated with 20 μM Dex (+Dex). One day later, half of the seedlings were inoculated with 50,000 spores/ml of *Hpa* isolate Emwa1 (Dex + Hpa) to activate RPP4. All seedlings were harvested 3 days after inoculation and stained with Trypan blue to visualize cell death. (D) Pictures of representative Trypan blue–stained cotyledons. (E) 20 cotyledons were evaluated per genotype and treatment. CD, cell death; <50% of cot., extensive cell death covering less than 50% of cotyledon; >50% of cot., extensive cell death covering more than 50% of cotyledon.

vious results showing that HR can occur in the absence of pathogen growth restriction and that HR can be inhibited by caspase inhibitors (6, 31). The pathogen-stress marker PR1 is not induced by *AtMC2-ΔN* overexpression, suggesting that these results are not a general consequence of stress. Finally, *AtMC2* control of *AtMC1* is post-transcriptional (fig. S7).

Our data establish *AtMC1* as a pro-death caspase-like protein required for both superoxide-dependent cell death in a reactive oxygen-sensitized state and for full HR mediated by intracellular NB-LRR immune receptor proteins. *AtMC2* antagonizes these functions. *AtMC1* and *AtMC2* contain a plant-specific N terminus shared with *LSD1*. The functions of both *AtMC1* and *AtMC2*

are enhanced by removal of this domain and, at least for *AtMC1*, this domain is required for interaction with *LSD1*. In the absence of *LSD1*, *AtMC1*-dependent cell death requires additional, unknown signals and is developmentally regulated. Hence, activation of *AtMC1* is complex and probably context-dependent. The inhibitory function of *AtMC2* does not require classic cysteine catalytic residues. The *AtMC1*-*AtMC2* antagonism is reminiscent of animal caspase-12, which negatively regulates caspase-1 to dampen the inflammatory response to bacteria and in colitis-associated colorectal cancer (32, 33); these caspase-12 functions do not require catalytic function (33). Caspase-12 also inhibits NOD-like receptor-mediated innate immunity independent of caspase-1 (34), providing a striking parallel to our observation that *AtMC2* inhibits *AtMC1*-dependent cell death controlled by analogous plant NB-LRR innate immune receptors. These results suggest an ancient link between cell death control by divergent metacaspase/caspase proteases and innate immune receptor function governed by NB-LRR or NLR proteins.



**Fig. 4.** *AtMC2* negatively regulates *AtMC1*. Seedlings of the depicted genotypes were sprayed with 1 μM Dex 6, 11, and 16 days after germination or left unsprayed. CACA denotes constructs where both putative catalytically active cysteine residues (C135A C256A for *AtMC2*) were mutated to alanine. (A) For ion leakage measurements, plants were sprayed with 150 μM BTH 1 day after the last treatment with Dex. Tissue was harvested at 43 hpt and processed as described in the SOM. Blue lines, Dex-treated genotypes; yellow lines, non-Dex-treated controls. Error bars represent two times the standard error. Letters a and b at right represent experimental groups with significant differences ( $P < 0.05$ , Tukey's HSD test). The experiment was repeated twice. (B) Plants were vacuum-infiltrated with 250,000 colony-forming units (CFU)/ml of *Pto* DC3000(*avrRpm1*). Twelve hours later, plants were harvested and stained with Trypan blue to visualize cell death. To quantify cell death, all dead cells in one field of vision (10× magnification) were counted. Average and two times the standard error were calculated from 20 leaves per genotype and treatment. The experiment was repeated. (C) Ten hours after the last treatment with Dex, plants of indicated genotypes were treated as in (B). (D) Seedlings were dip-infiltrated with *Pto* DC3000(*avrRpm1*) at  $2.5 \times 10^7$  CFU/ml. The average and two times the standard error were calculated from four samples per genotype. The experiment is representative of three independent replicates. (E) Three hours after the last Dex spray, plants were treated as in (D). (F) One day after the last Dex treatment, plants were inoculated with 50,000 spores/ml of *Hpa* Emw1. Three days later, plants were stained with Trypan blue, and interaction sites per field of vision were counted. The experiment was repeated three times. TN, trailing necrosis; FH, free hyphae.

#### References and Notes

1. E. Lam, *Nat. Rev. Mol. Cell Biol.* **5**, 305 (2004).
2. L. Aravind, E. V. Koonin, *Proteins* **46**, 355 (2002).
3. A. G. Uren *et al.*, *Mol. Cell* **6**, 961 (2000).
4. N. V. Chichkova *et al.*, *EMBO J.* **29**, 1149 (2010).
5. N. Hatsugai *et al.*, *Genes Dev.* **23**, 2496 (2009).
6. N. Hatsugai *et al.*, *Science* **305**, 855 (2004).
7. E. Lam, O. del Pozo, *Plant Mol. Biol.* **44**, 417 (2000).
8. E. Rojo *et al.*, *Curr. Biol.* **14**, 1897 (2004).
9. D. Vercammen, W. Declercq, P. Vandenabeele, F. Van Breusegem, *J. Cell Biol.* **179**, 375 (2007).
10. D. Vercammen *et al.*, *J. Biol. Chem.* **279**, 45329 (2004).
11. J. F. Sundström *et al.*, *Nat. Cell Biol.* **11**, 1347 (2009).
12. R. A. Dietrich *et al.*, *Cell* **77**, 565 (1994).
13. R. A. Dietrich, M. H. Richberg, R. Schmidt, C. Dean, J. L. Dangl, *Cell* **88**, 685 (1997).
14. D. H. Aviv *et al.*, *Plant J.* **29**, 381 (2002).
15. T. Jabs, R. A. Dietrich, J. L. Dangl, *Science* **273**, 1853 (1996).
16. C. Rustérucci, D. H. Aviv, B. F. Holt 3rd, J. L. Dangl, J. E. Parker, *Plant Cell* **13**, 2211 (2001).
17. A. Mateo *et al.*, *Plant Physiol.* **136**, 2818 (2004).
18. P. Mühlenbock, M. Plaszczka, E. Mellerowicz, S. Karpinski, *Plant Cell* **19**, 3819 (2007).
19. P. Mühlenbock *et al.*, *Plant Cell* **20**, 2339 (2008).
20. M. A. Torres, J. D. Jones, J. L. Dangl, *Nat. Genet.* **37**, 1130 (2005).
21. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr; and x, any amino acid.
22. H. Kaminaka *et al.*, *EMBO J.* **25**, 4400 (2006).
23. Materials and methods are available as supporting material on Science Online.
24. E. A. van der Biezen, C. T. Freddie, K. Kahn, J. E. Parker, J. D. Jones, *Plant J.* **29**, 439 (2002).
25. M. R. Grant *et al.*, *Science* **269**, 843 (1995).
26. P. V. Bozhkov *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 14463 (2005).
27. N. Watanabe, E. Lam, *J. Biol. Chem.* **280**, 14691 (2005).
28. B. Belenghi *et al.*, *J. Biol. Chem.* **282**, 1352 (2007).
29. A. Szallies, B. K. Kubata, M. Duszynski, *FEBS Lett.* **517**, 144 (2002).
30. Because this assay counts interaction sites, the number of spores that infect each leaf determines the maximum number of interactions that can occur per leaf. This cannot be altered by an increase in RPP4-dependent responses.



31. K. S. Century, E. B. Holub, B. J. Staskawicz, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 6597 (1995).
32. J. Dupaul-Chicoine *et al.*, *Immunity* **32**, 367 (2010).
33. M. Saleh *et al.*, *Nature* **440**, 1064 (2006).
34. P. M. LeBlanc *et al.*, *Cell Host Microbe* **3**, 146 (2008).
35. We thank J. McDowell (Virginia Tech, Blacksburg, VA) and M. Nishimura (UNC) for critical reading of the manuscript. This work was funded by NIH R01 GM057171 to J.L.D.; a Swiss National Science Foundation

Fellowship (PBEZA-115173) to N.S.C.; and the Research Fund, Ghent University (grant 12051403), and Fonds voor Wetenschappelijk Onderzoek-Vlaanderen to F.V.B. and D.V., respectively. We thank D. Baltrus for statistical analysis, T. Perdue of the UNC Biology Microscope Facility for patient and expert assistance, E. Washington and T. Eitas for pMDC7 vectors, B. van de Cotte for technical assistance, and K. Overmyer for early contributions to this work.

## Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1194980/DC1  
Materials and Methods  
Figs. S1 to S7  
References

12 July 2010; accepted 19 October 2010

Published online 18 November 2010;  
10.1126/science.1194980

# An Antagonistic Pair of *FT* Homologs Mediates the Control of Flowering Time in Sugar Beet

Pierre A. Pin,<sup>1,2</sup> Reyes Benlloch,<sup>1</sup> Dominique Bonnet,<sup>3</sup> Elisabeth Wremeth-Weich,<sup>2</sup> Thomas Kraft,<sup>2</sup> Jan J. L. Gielen,<sup>3</sup> Ove Nilsson<sup>1\*</sup>

Cultivated beets (*Beta vulgaris* ssp. *vulgaris*) are unable to form reproductive shoots during the first year of their life cycle. Flowering only occurs if plants get vernalized, that is, pass through the winter, and are subsequently exposed to an increasing day length (photoperiod) in spring. Here, we show that the regulation of flowering time in beets is controlled by the interplay of two paralogs of the *FLOWERING LOCUS T* (*FT*) gene in *Arabidopsis* that have evolved antagonistic functions. *BvFT2* is functionally conserved with *FT* and essential for flowering. In contrast, *BvFT1* represses flowering and its down-regulation is crucial for the vernalization response in beets. These data suggest that the beet has evolved a different strategy relative to *Arabidopsis* and cereals to regulate vernalization.

In flowering plants, the timing of the transition from the vegetative to the reproductive stage is determined by interactions between the developmental state of the plant and exo-

genous stimuli, such as changes in day length and temperature. Vernalization is a checkpoint through which many plants must pass, where flowering is conditional on prolonged exposure

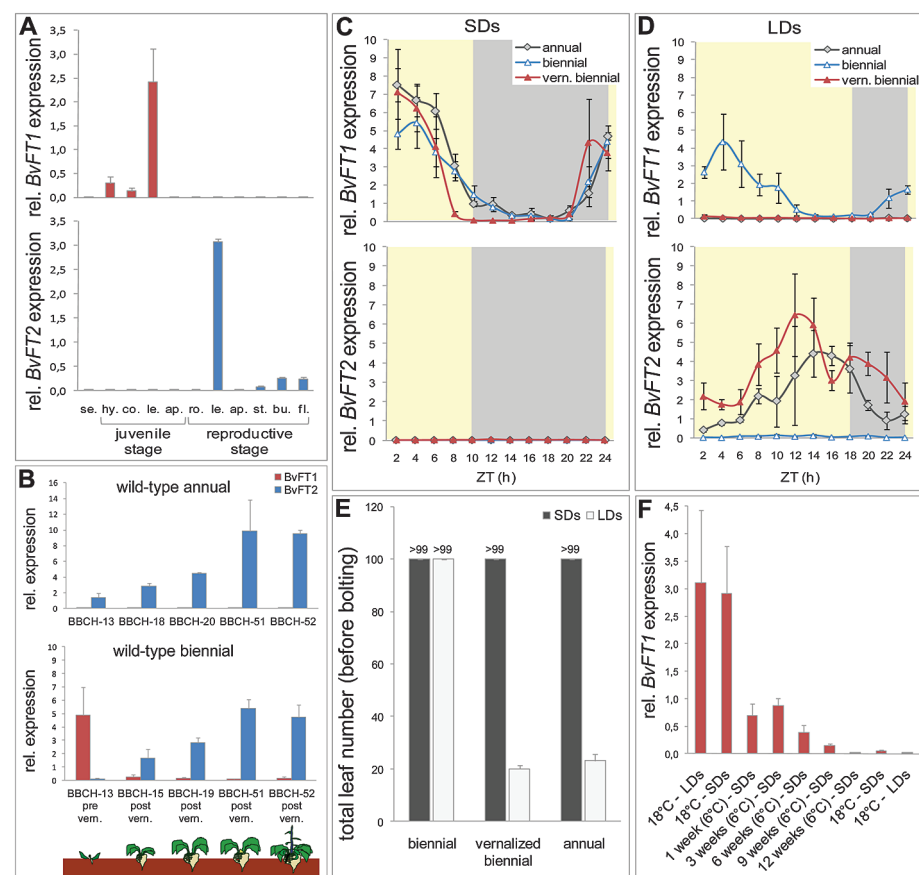
to cold temperature and is subsequently induced by other inductive pathways (1). Vernalization of the winter-annual *Arabidopsis* and other members of the *Brassicaceae* family involve several steps that culminate in the repression of the major floral inhibitor gene *FLOWERING LOCUS C* (*FLC*) (2–5). In cereals, cold temperature stimuli are integrated by the activation of the *FRUIT-FULL* (*FUL*)/*APETALA1* (*API*) homolog *VRN1* (6), which represses the *CONSTANS*, *CONSTANS*-like, and *TIMING OF CAB EXPRESSION 1* (*CCT*)-domain transcription factor *VRN2* (7, 8).

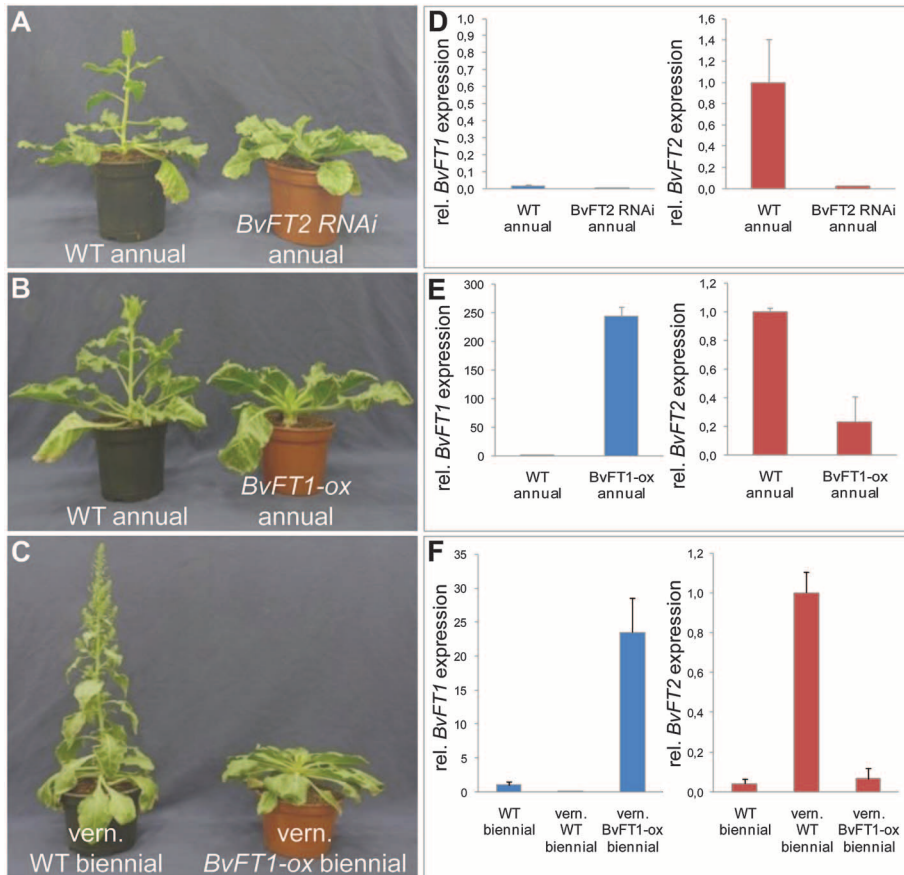
The phosphatidylethanolamine-binding protein (*PEBP*) gene family, found in both angiosperms and gymnosperms (9), has evolved both activators and repressors of flowering. The *Arabidopsis*

<sup>1</sup>Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, 901-83 Umeå, Sweden. <sup>2</sup>Syngenta Seeds AB, Box 302, 261-23 Landskrona, Sweden. <sup>3</sup>Syngenta Seeds SAS, 12 Chemin de l'Hôbit, 31790 Saint-Sauveur, France.

\*To whom correspondence should be addressed. E-mail: Ove.Nilsson@genfys.slu.se

**Fig. 1.** Expression pattern of *BvFT1* and *BvFT2* in beets (15). (A) Expression of *BvFT1* and *BvFT2* in various tissues, including se., seed; hy., hypocotyl; co., cotyledon; le., leaf; ap., apex; ro., root; st., stem; bu., floral bud, and fl., flower. Samples were harvested in LDs at zeitgeber time (ZT) 8 (i.e., 8 hours after lights on). Error bars, mean  $\pm$  SE ( $n = 3$ ). (B) Expression of *BvFT1* and *BvFT2* in leaf samples in LDs across different developmental stages in annuals and biennials. Samples were harvested at ZT6. Error bars, mean  $\pm$  SE ( $n = 3$ ). (C and D) Diurnal rhythms of *BvFT1* and *BvFT2* in annual, biennial, and vernalized-biennial beets in SDs (C) and LDs (D). Error bars, mean  $\pm$  SE ( $n = 5$ ). (E) Leaf number at time of bolting in biennial, vernalized-biennial, and annual plants grown under the different photoperiodic conditions described in (C) and (D). Error bars, mean  $\pm$  SD ( $n = 6$ ). (F) *BvFT1* transcript accumulation in biennials in response to vernalization. Leaf samples were harvested at ZT6. Error bars, mean  $\pm$  SE ( $n = 3$ ).





**Fig. 2.** Misexpression of *BvFT1* or *BvFT2* control bolting in sugar beet. (A to C) Nonbolting phenotype observed in *BvFT2 RNAi* annual (A), *BvFT1-ox* annual (B), and vernalized *BvFT1-ox* biennial (C) plants when grown in LDs for 6 weeks. (D to F) *BvFT1* and *BvFT2* expression in *BvFT2 RNAi* (D), *BvFT1-ox* (E), and vernalized *BvFT1-ox* (F) plants (15). Expression levels were set to unity in wild type (WT). Error bar, mean  $\pm$  SE ( $n = 3$ ).

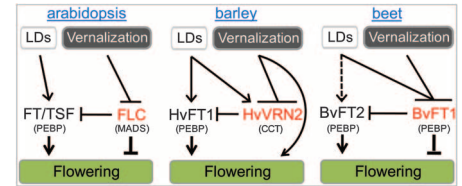
PEBP gene *FLOWERING LOCUS T* (*FT*) (10, 11) is a major output of the photoperiod pathway and controls the floral transition in response to changes in day length, whereas its close homolog *TERMINAL FLOWER 1* (*TFL1*) acts as a strong floral repressor (10–12).

Cultivated sugar beet (*Beta vulgaris* ssp. *vulgaris*) is a biennial crop that requires vernalization to enter the generative phase. The vernalization period typically needs to be followed by exposure to long days (LDs). Without LDs, floral induction does not occur in beets, despite the fact that they become competent to flower after vernalization (13). One of the main factors controlling flowering time in beets is the bolting gene *B*, a dominant promoter of flowering that overrides the need for vernalization (14). Plants carrying the dominant *B* allele behave as annuals and proceed to bolting and flowering as a direct response to LDs.

To determine whether *FT* function is conserved between beets and *Arabidopsis*, we initiated a first characterization of the PEBP genes in *Beta* (15), isolating two genes grouped within the *FT*-like clade [*BvFT1* and *BvFT2* (fig. S1A)]. Genetic mapping showed that none of the PEBP homologs maps in vicinity to *B* (fig. S2) on chromosome II (16). Complementation experiments

and gene expression analysis showed that *BvFT2* is the functional *FT* ortholog in beets. Transgenic expression of *BvFT2* in both *Arabidopsis* and sugar beet (fig. S3, B and G, and fig. S4) strongly promoted flowering. Furthermore, *BvFT2* expression levels were correlated with the initiation of flowering in both annual and biennial beets (Fig. 1B). When *BvFT2* expression was suppressed by RNA interference (*RNAi*) in annual plants, a continuous vegetative growth was observed in LDs (Fig. 2A and fig. S5A), suggesting that *BvFT2* is essential for flowering. Unexpectedly, *BvFT1* repressed flowering when ectopically expressed in transgenic sugar beet and *Arabidopsis* plants (Fig. 2, B and C, fig. S5, B and C, and fig. S3, C and G). These data suggest that an *FT*-like gene, evolutionarily more related to *FT* than to *TFL1/CEN* (figs. S1A and S6), has evolved into a true flowering repressor. *BvFT1* expression was not affected in the *BvFT2 RNAi* plants (Fig. 2D), whereas *BvFT2* expression was dramatically reduced compared with wild type in *BvFT1-ox* plants, both before (Fig. 2E) and after (Fig. 2F) vernalization.

In sunflower, neofunctionalization (the evolution of new function and/or expression of a duplicated gene) (17) of *FT* function was postulated to have occurred as a result of a frame-



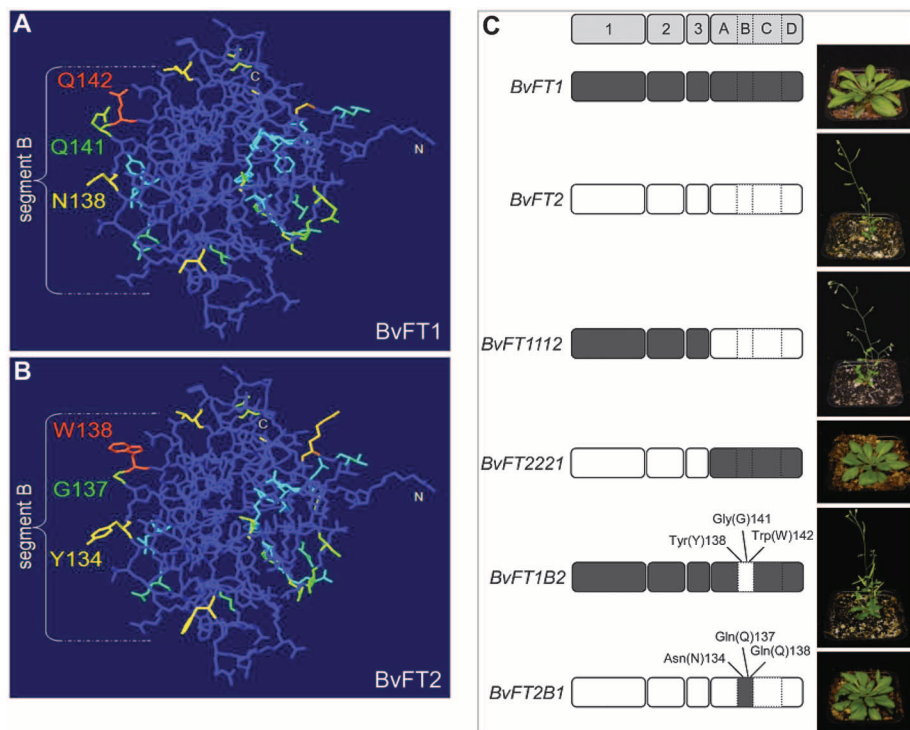
**Fig. 3.** Simplified flowering models in beets, *Arabidopsis*, and barley, showing the key integrators of the vernalization pathway, which mediate transcriptional regulation of the *FT* orthologs.

shift mutation in an *FT*-like gene, ensuing in the *HaFT1* gene acquiring a role in delaying flowering through a dominant-negative interference with an activating paralogue, *HaFT4* (18) (fig. S6). This does not appear to be the case for *BvFT1*, which acts as a true repressor on the basis of the *Arabidopsis* overexpression phenotypes (fig. S3, J and K), the differences between the *BvFT2 RNAi* and *BvFT1-ox* phenotypes in beets (Fig. 2 and fig. S5), and the fact that expression of *BvFT1* and *BvFT2* is mutually exclusive rather than overlapping (Fig. 1, A, C, and D).

As for all previously characterized *FT*-like genes, transcripts of both *BvFT1* and *BvFT2* were mainly detected in leaves (Fig. 1A), but their respective temporal expression patterns were very different. Whereas *BvFT1* was expressed in the leaves (Fig. 1A) of plants that were not competent to flower, that is, plants grown in short days (SDs) or nonvernalized biennials (Fig. 1, C and D), *BvFT2* expression always correlated with flower induction and reproductively mature plants (Fig. 1, A to D). These observations suggest that neofunctionalization of the pair of *Beta FT* paralogs occurred in terms of both expression profiles and protein function. Expression of *BvFT1* and *BvFT2* displays diurnal oscillations (Fig. 1, C and D), suggesting that both genes might be under the control of the circadian clock. However, *BvFT1* is preferentially expressed in the morning in SDs (Fig. 1C), whereas *BvFT2* is expressed in the evening and in LDs (Fig. 1D). In annuals, expression of both genes responded quickly to changes in photoperiod, but in opposing ways (fig. S7). Transcription of *BvFT1* was not repressed in LDs in biennials before vernalization, and *BvFT2* expression was not induced (Fig. 1D). This suggests that biennials might be insensitive to the photoperiodic signal before vernalization and that a vernalization-dependent factor acts to restore the photoperiodic regulation of *BvFT1* and *BvFT2*. Consequently, these observations suggest that *BvFT1* and *BvFT2* regulation is dependent on the presence of the dominant allele *B*.

Vernalization and photoperiod pathways are connected by the coordinated repression of *BvFT1*. *BvFT1* transcription in biennials was both reduced during vernalization (Fig. 1F) and maintained after winter at low levels if plants were grown in LDs. These data indicate that cold treatment induces *BvFT1* repression and also affects the vernalization-dependent factors that maintain *BvFT1* repression in LDs. Given that a transient





**Fig. 4.** Characterization of important regions of BvFT1 and BvFT2 involved in their antagonistic functions. **(A and B)** Folding prediction of BvFT1 and BvFT2. The structures are shown as solid three-dimensional traces with an alignment diversity color setting. **(C)** Chimeric FT proteins containing domains of BvFT1 and BvFT2 show antagonistic function when expressed in *Arabidopsis*.

exposure to SDs directly after vernalization [so called “devernalization,” a condition known to abolish flowering in *Beta* (19)] resulted in the restoration of BvFT1 expression (fig. S8), unfavorable conditions for flowering appear to be coupled to expression of BvFT1, which suggests that BvFT1 is important for the devernalization phenomenon.

On the basis of these results, we propose a model whereby BvFT1 prevents flowering during SDs and before vernalization by repressing the expression of BvFT2 (Fig. 3). Biennials, lacking *B*, would be unable to sense the long-day signal before vernalization because of high levels of BvFT1 expression. During vernalization, BvFT1 expression decreases and the plant becomes competent to respond to LDs, which is required to maintain low levels of BvFT1 expression. This appears to be necessary to induce flowering. However, formally, we cannot exclude that other factors might also contribute to the vernalization response and the regulation of BvFT2 (Fig. 3, dotted arrow).

Unexpectedly, BvFT2 RNAi plants responded to vernalization and bolted after cold treatment (fig. S9A), indicating that additional mechanisms are acting to promote bolting in beets in parallel to BvFT2. Although BvFT2 RNAi plants proceeded to bolting, flowering was completely abolished or initiated at very late stages (fig. S9, B and C), suggesting that BvFT2 is needed for a normal flower initiation. In contrast, because BvFT1-ox plants do not respond to vernalization followed by LDs (Fig. 2C and fig. S5C),

BvFT1 itself is likely to regulate downstream bolting and flowering promoters. Such a scenario suggests that in beets an FT-like gene represses bolting and flowering as part of the vernalization response (Fig. 3). In *Arabidopsis*, *FLC* inhibits flowering, whereas in cereals, *VRN2* (a member of the CCT gene family) performs this role (Fig. 3). This suggests that the vernalization response has developed differently not only in monocots and eudicots but also in the two eudicot families *Brassicaceae* and *Amaranthaceae* (formerly *Chenopodiaceae*) within the evolutionarily diverged eudicot groups of the Rosids and Caryophyllales, respectively. Sequence comparison between the two proteins showed that both BvFT1 and BvFT2 carry the functionally important FT signatures, Tyr85(Y) and Gln140(Q) (fig. S1C) (20). However, the proteins differ within the segment B of the fourth exon [encoding an external loop of PEBP (21)] (Fig. 4, A and B, and fig. S1C), which is important for FT versus TFL1 function in *Arabidopsis* (21). BvFT2 is identical in this region to most other FTs (consistent with its role in promoting flowering), whereas BvFT1 has three substitutions out of 14 amino acids (Fig. 4A and fig. S1C). By swapping regions between BvFT1 and BvFT2 (Fig. 4C and table S1), we demonstrated that divergence within these three amino acids of the external loop is the major cause of their antagonistic functions. Remarkably, expression of BvFT1B2 (chimeric BvFT1 carrying the external loop of BvFT2 with the three altered amino acids) can

completely revert its repressing function to promoting function (Fig. 4C and table S1). This suggests that BvFT1 was initially a promoter of flowering but that mutations within the external loop of its protein resulted in a shift in function to flowering repression. We are currently investigating which of the three amino acids are critical for the activator versus repressor function.

To get further insights into the evolution of the BvFT1 repressor function, we isolated BvFT1- and BvFT2-like sequences from other *Beta* species, as well as other members of the *Amaranthaceae* family. BvFT2-like genes showed high homology across the *Amaranthaceae* (fig. S10, A and B). In contrast, we could only isolate BvFT1-like sequences from *Beta* species, suggesting that FT1 arose through gene duplication during the diversification of the genus *Beta*. We cannot exclude that FT1 may be present in other *Amaranthaceae*, but given the high level of conservation of FT-like genes, our results suggest that FT1 is likely to be restricted to *Beta*. Gene expression analysis showed that in LDs FT1 was expressed in all cultivated varieties of *B. vulgaris*, but not in wild *Beta* species, except in some *B. vulgaris* ssp. *maritima* accessions from northern latitudes (fig. S10C). In contrast, FT2 was expressed in these species, consistent with their flowering behavior. Indeed, *B. macrocarpa*, *B. precumbens*, and *B. vulgaris* ssp. *maritima* accessions from southern latitudes bolt and flower quickly without exposure to cold temperature, while *maritima* accessions from northern latitudes and all cultivated beets require vernalization to enter the generative phase (22). These results are consistent with our findings in sugar beet, clearly linking the BvFT1 expression in LDs to the biennial growth behavior. Interestingly, domesticated beets (ssp. *vulgaris*) most likely originated from *B. vulgaris* ssp. *maritima* plants (23).

Because BvFT1 overexpression leads to the prevention of bolting and flowering, even after a prolonged vernalization period (Fig. 2C and fig. S5C), it may have interesting applications in breeding. Our BvFT1-ox plants may fulfill the desire for a “winter beet” that can be planted in the fall without bolting during the following spring to provide a prolonged growing season and harvesting campaign with increased yields. This demonstrates how extending our understanding of the molecular determinants of vernalization has direct applications in agriculture.

#### References and Notes

1. S. Sung, R. M. Amasino, *Curr. Opin. Plant Biol.* **7**, 4 (2004).
2. S. D. Michaels, R. M. Amasino, *Plant Cell* **11**, 949 (1999).
3. C. C. Sheldon et al., *Plant Cell* **11**, 445 (1999).
4. M. Tadege et al., *Plant J.* **28**, 545 (2001).
5. R. Wang et al., *Nature* **459**, 423 (2009).
6. L. Yan et al., *Proc. Natl. Acad. Sci. U.S.A.* **100**, 6263 (2003).
7. L. Yan et al., *Science* **303**, 1640 (2004).
8. M. N. Hemming, W. J. Peacock, E. S. Dennis, B. Trevaskis, *Plant Physiol.* **147**, 355 (2008).
9. N. Gyllenstrand, D. Clapham, T. Källman, U. Lagercrantz, *Plant Physiol.* **144**, 248 (2007).
10. I. Kardailsky et al., *Science* **286**, 1962 (1999).
11. Y. Kobayashi, H. Kaya, K. Goto, M. Iwabuchi, T. Araki, *Science* **286**, 1960 (1999).

12. D. Bradley, O. Ratcliffe, C. Vincent, R. Carpenter, E. Coen, *Science* **275**, 80 (1997).
13. G. D. H. Bell, A. B. Bauer, *J. Agric. Sci.* **32**, 112 (1942).
14. F. A. Abegg, *J. Agric. Res.* **53**, 493 (1936).
15. Materials and methods are available as supporting material on Science Online.
16. P. Boudry et al., *Theor. Appl. Genet.* **88**, 852 (1994).
17. S. Ohno, *Evolution by Gene Duplication* (Springer-Verlag, New York, ed. 1, 1970).
18. B. K. Blackman, J. L. Strasburg, A. R. Raduski, S. D. Michaels, L. H. Rieseberg, *Curr. Biol.* **20**, 629 (2010).
19. H. Van Dijk, *J. Exp. Bot.* **60**, 3143 (2009).
20. Y. Hanzawa, T. Money, D. Bradley, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 7748 (2005).
21. J. H. Ahn et al., *EMBO J.* **25**, 605 (2006).
22. J. P. W. Letschert, thesis, *Beta Section Beta: Biogeographical Patterns of Variation and Taxonomy* (Wageningen Agricultural University Papers, 93-1, Wageningen, Netherlands 1993).
23. C. Jung, K. Pillen, L. Frese, S. Fähr, A. E. Melchinger, *Theor. Appl. Genet.* **86**, 449 (1993).
24. We thank F. Domène, J. Bensefelt, Å. Karlsson, R. Sant, P. van Roggen, and A.-M. Nilsson for technical assistance, and Y. Fischer for help in preparing the manuscript. We also thank C. Bellini for critical reading of the manuscript. This work was supported by the Swedish Research Council and the Swedish Governmental Agency for Innovation Systems to O.N. and T.K., as well as by Südzucker. Phylogenetic data have been deposited with Dryad Digital Repository (DOI: 10.5061/dryad.4930).

Nucleotide sequences have been deposited with GenBank under accession numbers HM448909 to HM448918 and HQ148107 to HQ148132. The use of *BvFT1* and *BvFT2* in control of sugar beet flowering is the subject of the patent application WO2010/025888.

#### Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6009/1397/DC1  
Materials and Methods  
SOM Text  
Figs. S1 to S10  
Tables S1 to S3  
References

26 August 2010; accepted 22 October 2010  
10.1126/science.1197004

# Alleviating Neuropathic Pain Hypersensitivity by Inhibiting PKM $\zeta$ in the Anterior Cingulate Cortex

Xiang-Yao Li,<sup>1,2\*</sup> Hyoung-Gon Ko,<sup>3\*</sup> Tao Chen,<sup>1,2\*</sup> Giannina Descalzi,<sup>1</sup> Kohei Koga,<sup>1</sup> Hansen Wang,<sup>1</sup> Susan S. Kim,<sup>1</sup> Yuze Shang,<sup>1</sup> Chuljung Kwak,<sup>3</sup> Soo-Won Park,<sup>3</sup> Jaehoon Shim,<sup>2,3</sup> Kyungmin Lee,<sup>3,4</sup> Graham L. Collingridge,<sup>2,5</sup> Bong-Kiun Kaang,<sup>2,3†</sup> Min Zhuo<sup>1,2†</sup>

Synaptic plasticity is a key mechanism for chronic pain. It occurs at different levels of the central nervous system, including spinal cord and cortex. Studies have mainly focused on signaling proteins that trigger these plastic changes, whereas few have addressed the maintenance of plastic changes related to chronic pain. We found that protein kinase M zeta (PKM $\zeta$ ) maintains pain-induced persistent changes in the mouse anterior cingulate cortex (ACC). Peripheral nerve injury caused activation of PKM $\zeta$  in the ACC, and inhibiting PKM $\zeta$  by a selective inhibitor,  $\zeta$ -pseudosubstrate inhibitory peptide (ZIP), erased synaptic potentiation. Microinjection of ZIP into the ACC blocked behavioral sensitization. These results suggest that PKM $\zeta$  in the ACC acts to maintain neuropathic pain. PKM $\zeta$  could thus be a new therapeutic target for treating chronic pain.

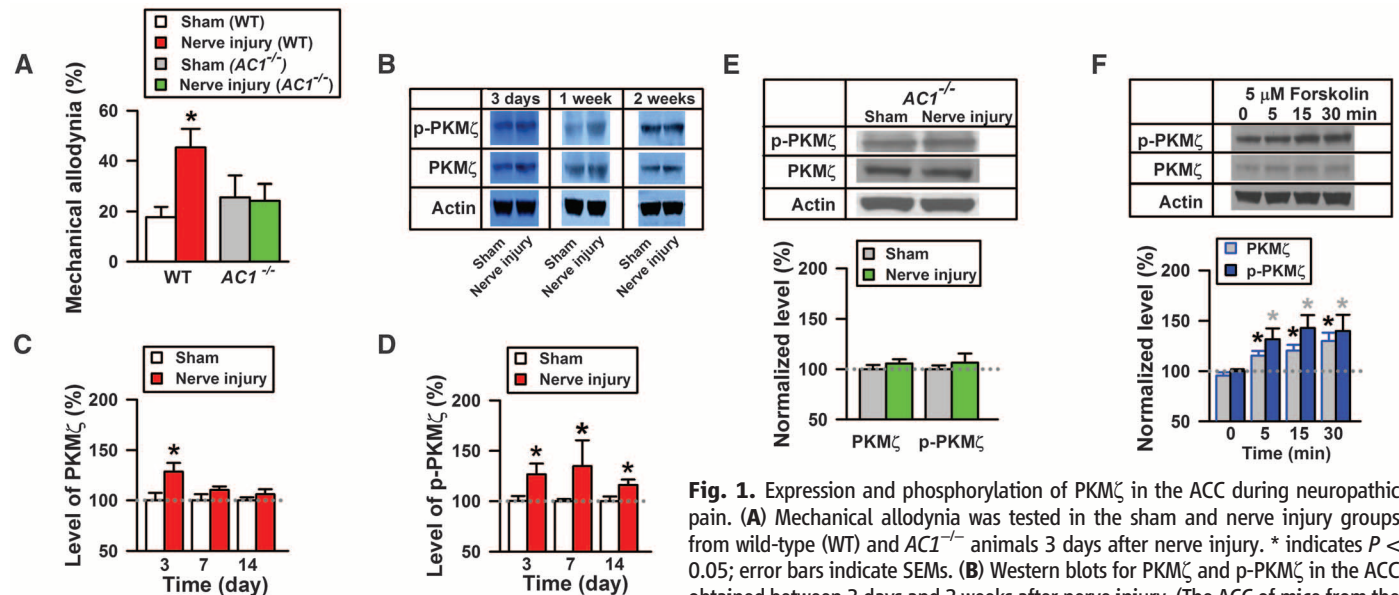
One key function of the brain is to learn and memorize sensory experiences and then adapt subsequent behavioral responses.

Investigations into the molecular mechanisms of such processes provide information for our basic understanding of brain functions (1). Synaptic

plasticity is the major cellular model used for understanding these mechanisms. In addition to numerous studies on synaptic mechanisms for learning and memory, these same synaptic mechanisms may be responsible for pathological pain conditions (2–4). For example, nerve injury triggers long-term plastic changes along sensory pathways, from the peripheral sensory terminals to the spinal cord dorsal horn, amygdala, and cortex (3–7). These plastic changes contribute to pain perception, fear, and memory. The persistent nature

<sup>1</sup>Department of Physiology, Faculty of Medicine, Center for the Study of Pain, University of Toronto, 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada. <sup>2</sup>Department of Brain and Cognitive Sciences, Seoul National University, Seoul 151-747, Korea. <sup>3</sup>National Creative Research Initiative Center for Memory, Department of Biological Sciences, College of Natural Sciences, Seoul National University, San 56-1 Silim-dong, Gwanak-gu, Seoul 151-747, Korea. <sup>4</sup>Department of Anatomy, School of Medicine, Kyungpook National University, 2-101 Dongin-Dong, Daegu 700-422, Korea. <sup>5</sup>Medical Research Council (MRC) Centre for Synaptic Plasticity, School of Physiology and Pharmacology, University of Bristol, Bristol BS8 1TD, UK.

\*These authors contributed equally to this work.  
†To whom correspondence should be addressed. E-mail: kaang@snu.ac.kr (B.-K.K.); min.zhuo@utoronto.ca (M.Z.)



**Fig. 1.** Expression and phosphorylation of PKM $\zeta$  in the ACC during neuropathic pain. (A) Mechanical allodynia was tested in the sham and nerve injury groups from wild-type (WT) and AC1<sup>-/-</sup> animals 3 days after nerve injury. \* indicates  $P < 0.05$ ; error bars indicate SEMs. (B) Western blots for PKM $\zeta$  and p-PKM $\zeta$  in the ACC obtained between 3 days and 2 weeks after nerve injury. (The ACC of mice from the sham and nerve injury groups were used for Western blot analysis 90 min after the allodynia test.) (C) Level of PKM $\zeta$  in the ACC of mice from the sham and nerve injury groups. Level of PKM $\zeta$  increased significantly 3 days after nerve injury compared with PKM $\zeta$  in the sham group. (D) Level of p-PKM $\zeta$  increased significantly 3 days after nerve injury. This effect lasted over 2 weeks after nerve injury compared with p-PKM $\zeta$  in the sham group. (E) Peripheral nerve injury did not increase the levels of PKM $\zeta$  and p-PKM $\zeta$  in the ACC of AC1<sup>-/-</sup> mice. (F) Forskolin incubation increased PKM $\zeta$  (gray) and p-PKM $\zeta$  (blue) levels in the ACC of WT mice.



of chronic pain results in resistance to commonly used analgesics (6). Therefore, revealing the molecular mechanisms that are involved in the main-

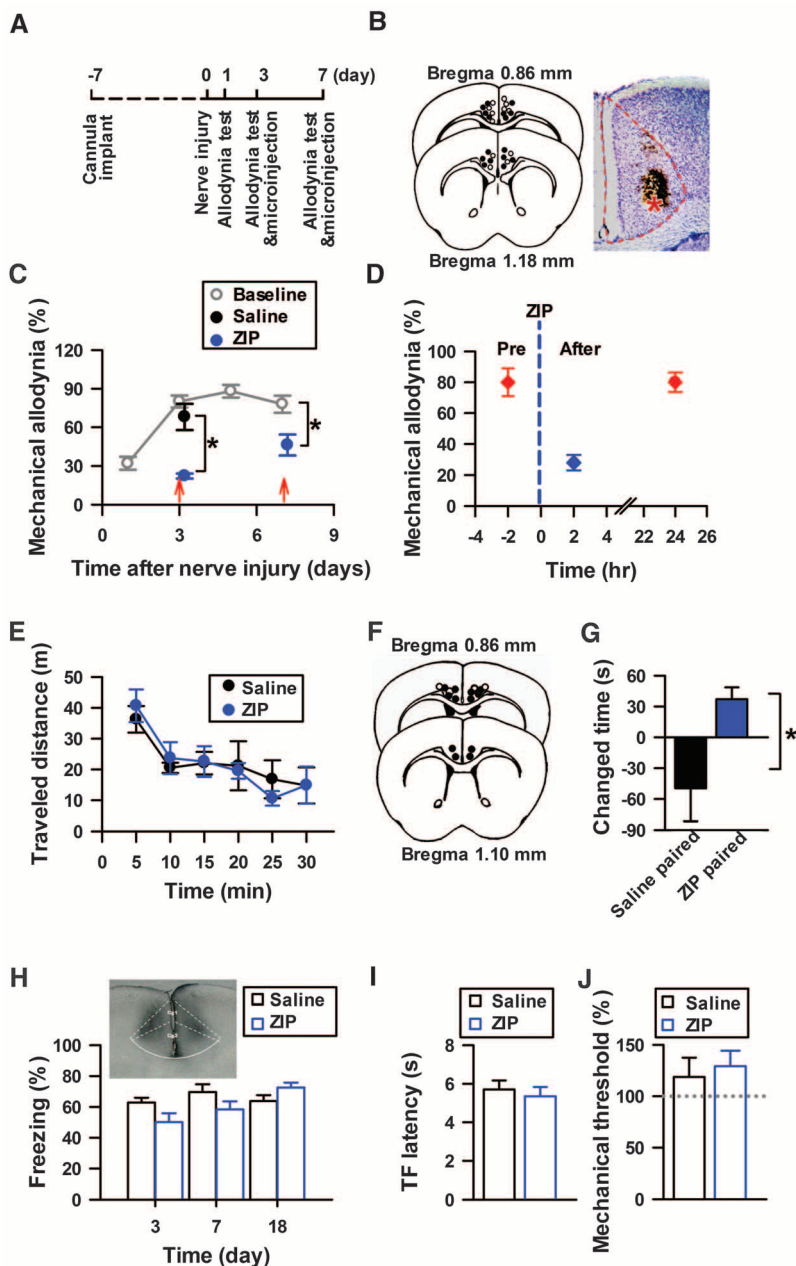
tenance of pain-related long-term synaptic plasticity changes is critical for developing effective chronic pain treatments.

Multiple protein kinases are thought to contribute to the induction of long-term potentiation (LTP) and initial consolidation of information storage. Among them, only protein kinase M zeta (PKM $\zeta$ ) maintains persistent synaptic changes (8–10). PKM $\zeta$ , an atypical isoform of protein kinase C (PKC), has been detected in many regions of the brain, including the frontal cortex (11). In the hippocampus, LTP induction triggers the synthesis of PKM $\zeta$ , and activation of PKM $\zeta$  is critical for late-phase LTP (L-LTP) and memory consolidation (8, 12–15).

The anterior cingulate cortex (ACC) is a key cortical area involved in chronic pain (2, 16, 17). We first examined whether, in the ACC, peripheral nerve injury causes changes in PKM $\zeta$  (18). As reported previously (17, 19), behavioral allodynic response was increased 3 days after nerve injury when compared with the response in sham-treated mice (Fig. 1A). The levels of PKM $\zeta$  in the ACC were significantly increased after nerve injury (sham-operated versus nerve injury group;  $n = 9$  mice for each group, unpaired  $t$  test,  $t_{16} = -2.51$ ,  $P < 0.05$ ; Fig. 1, B and C). Because PKM $\zeta$  is activated by phosphorylation, we also conducted experiments to detect possible changes in the level of phosphorylated PKM $\zeta$  (p-PKM $\zeta$ ). Consistently, the level of p-PKM $\zeta$  was also significantly increased ( $n = 8$  for each group, unpaired  $t$  test,  $t_{14} = -2.26$ ,  $P < 0.05$ ; Fig. 1, B and D). These data suggest that peripheral nerve injury increases PKM $\zeta$  activity in the ACC.

To determine whether such changes are long-lasting, we examined PKM $\zeta$  and p-PKM $\zeta$  levels 7 and 14 days after nerve injury. Although behavioral allodynia persisted at these time points, the protein levels of PKM $\zeta$  returned to baseline (Fig. 1C), indicating that the regulation of the amount of PKM $\zeta$  is short-lasting. However, the p-PKM $\zeta$  level remained increased [two-way analysis of variance (ANOVA),  $F_{1,28} = 6.10$ ,  $P < 0.05$ ; Fig. 1D], suggesting that PKM $\zeta$  activity could contribute to the maintenance of neuropathic pain. To investigate whether the changes in PKM $\zeta$  protein levels or activity were a generalized phenomenon in the central nervous system, we also examined the levels of PKM $\zeta$  and p-PKM $\zeta$  in the hippocampus and spinal dorsal horn 3 days after nerve injury. PKM $\zeta$  and p-PKM $\zeta$  levels in the hippocampus and spinal cord did not change after nerve injury (fig. S1), suggesting that changes in PKM $\zeta$  are affected in a regionally specific manner in response to peripheral nerve injury.

What could be the possible second messengers that trigger the activation and synthesis of PKM $\zeta$  in the ACC? In the ACC, calmodulin-stimulated adenylyl cyclase 1 (AC1) is critical for ACC LTP and behavioral sensitization caused by peripheral nerve injury (16, 20). Thus, we examined whether AC1 acts upstream of PKM $\zeta$  in the ACC after nerve injury by using AC1 gene knockout mice ( $AC1^{-/-}$ ).  $AC1^{-/-}$  mice did not show any significant behavioral sensitization 3 days after nerve injury (Fig. 1A). Similar amounts



**Fig. 2.** Bilateral microinjections of ZIP into the ACC decreased allodynia in mice with peripheral nerve injury. (A) The schematic view of the microinjection experiments. (B) Location of microinjection sites in the ACC. Solid circles represent ZIP injection sites, whereas open circles represent saline injection sites. The right image shows an example of the location of the cannula tip in one ACC slice with hematoxylin and eosin staining. The injection site is indicated by an asterisk. (C) Microinjection of ZIP (blue) into the ACC decreased the positive response rate in the nerve injury animal, whereas saline microinjection (black) into the ACC had no effect on the response level. Open gray circles represent the summary control data of positive response level tested from the saline and ZIP group. The red arrows indicated the time point of ZIP injection.  $*P < 0.05$ ; error bars, SEMs. (D) Two hours after injection, ZIP still has an analgesic effect. However, the allodynia response recovered to preinjection level 24 hours after injection. (E) ZIP has no effect on the traveled distance in the open field test. (F) The location of microinjection sites in the ACC in the conditioned place preference experiments. Solid or open circles indicate the location of the injection that did or did not induce place preference, respectively. (G) The changed time in the ZIP paired chamber was significantly different from that of the saline paired chamber.  $*P < 0.05$ . (H) The effect of ZIP infusion into the ACC on contextual fear memory. Representative brain slice shows the location of the injection site. ZIP was infused into the ACC 2 hours before memory retrieval, which was tested 3, 7, and 18 days after conditioning. (I) ZIP did not change the response latency in the tail-flick (TF) test. (J) Microinjection of ZIP into the ACC had no effect on the paw-withdrawal threshold in normal mice.

of PKM $\zeta$  and p-PKM $\zeta$  were found in sham-operated and nerve-injured *AC1*<sup>-/-</sup> mice 3 days after nerve injury ( $n = 9$  for each group, unpaired  $t$  test, PKM $\zeta$ ,  $t_{16} = -0.99$ , p-PKM $\zeta$ ,  $t_{16} = -0.67$ ,  $P > 0.05$ , Fig. 1E). To further examine the link between the AC1 and PKM $\zeta$ , we performed brain slice experiments. We used bath application of forskolin (5  $\mu$ M), a cell-permeable activator of AC. Forskolin treatment increased the amount of PKM $\zeta$  and p-PKM $\zeta$  in a time-dependent manner (Fig. 1F). The increase in PKM $\zeta$  was rapid; the amount of PKM $\zeta$  was increased within 5 min after forskolin (5  $\mu$ M) treatment (one-way ANOVA,  $F_{3,15} = 4.52$ ,  $P < 0.05$ ,  $n = 7$  per group; Fig. 1F). This suggests that adenosine 3',5'-monophosphate (cAMP) signaling increases PKM $\zeta$  levels in a transcription-independent manner, because transcription of PKM $\zeta$  pre-mRNA is likely to take more than 30 min (12, 21).

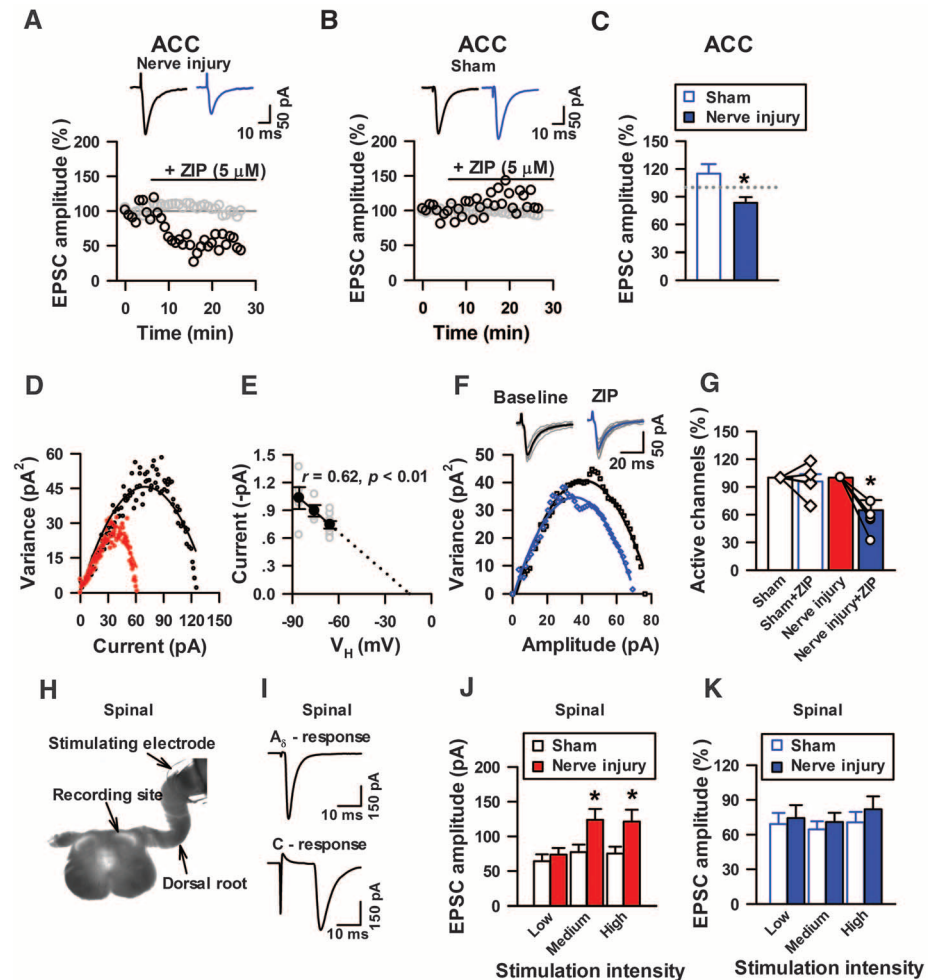
So what could be the function of increased PKM $\zeta$  activity in the ACC? We wondered whether this increase contributes to behavioral sensitization after nerve injury. We performed intra-ACC microinjections of  $\zeta$ -pseudosubstrate inhibitory peptide (ZIP) (10 nmol/ $\mu$ l, 0.5  $\mu$ l per side), a selective cell-permeable PKM $\zeta$  inhibitor, in mice 3 days after nerve injury. Bilateral microinjections of ZIP into the ACC significantly reduced the allodynia ( $n = 8$ , paired  $t$  test,  $t_7 = 8.78$ ,  $P < 0.001$ ; Fig. 2, A to C) unlike saline-injected controls ( $n = 6$ , paired  $t$  test,  $t_5 = 1.84$ ,  $P > 0.05$ ; Fig. 2C). Similar analgesic effects of ZIP were detected 7 days after nerve injury ( $n = 7$ , paired  $t$  test,  $t_6 = 2.89$ ,  $P < 0.05$ ; Fig. 2C). The analgesic effects lasted for at least 2 hours, but there was recovery at 24 hours after the injection ( $n = 5$ , Fig. 2D). To determine whether the effect of ZIP is regionally selective, we microinjected ZIP into the primary somatosensory cortex. The same amount of ZIP did not produce any significant analgesic effect (paired  $t$  test,  $t_3 = -2.30$ ,  $P > 0.05$ ,  $n = 4$ , fig. S2, A and B). To determine whether the effects of ZIP in ACC are sensory-selective, we examined the effects of this inhibitor in the open-field test and found no differences in the distance traveled between mice microinjected with ZIP or saline (Fig. 2E).

Nerve injury can induce tonic pain (22). By using an injury-induced, conditioned place preference paradigm (22), we evaluated the effects of ZIP on nerve injury-related tonic pain. Microinjection of ZIP into the ACC also produced significant analgesic effects ( $n = 6$ , paired  $t$  test,  $t_5 = -3.19$ ,  $P < 0.05$ ; Fig. 2, F and G). In addition, we examined whether ZIP infusion caused any memory impairment. When we infused the ACC with ZIP 2 hours before memory retrieval on 3, 7, or 18 days after contextual fear conditioning, we did not find any obvious defects in memory retrieval [ $n = 7$  to 11 per group, unpaired  $t$  test,  $P > 0.05$  in day 3 ( $t_{20} = 1.92$ ), 7 ( $t_{16} = 1.56$ ) or 18 ( $t_{13} = -1.75$ ); Fig. 2H]. Moreover, microinjecting ZIP into the ACC did not affect the tail-flick reflex and the paw-withdrawal threshold in normal animals (Fig. 2, I and J). These data show that microinjection of ZIP into the ACC produces a modality

and regionally selective analgesic effect on nerve injury-related tonic pain.

What is likely to be the synaptic mechanism responsible for the analgesic effects produced by ZIP in neuropathic pain? PKM $\zeta$  can potentiate postsynaptically the amplitude of  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA)

receptor-mediated excitatory postsynaptic currents (EPSCs) (23). Because glutamatergic synaptic transmission in the ACC is increased after nerve injury (17), we speculated that PKM $\zeta$  may contribute to the maintenance of enhanced synaptic transmission induced by nerve injury. First, we recorded AMPA receptor-mediated EPSCs in layers II and



**Fig. 3.** Inhibition of PKM $\zeta$  selectively decreased the amplitude of eEPSCs in ACC neurons of mice with neuropathic pain by reducing the number of active AMPARs. (A and B) Samples showed the effect of ZIP (5  $\mu$ M) on the amplitude of eEPSCs in the ACC neurons of animals from the nerve injury [black circles in (A)] and sham group [black circles in (B)]. The gray open circles represent the change of membrane resistance during recording. Black traces in the upper part of (A) and (B) indicate the averaged response at baseline, and blue traces indicate the average of 2 min responses collected 10 min after ZIP application. (C) Pooled data of effects of ZIP on the eEPSCs recorded from the ACC of mice in the sham (open) and nerve injury (solid) groups 10 min after ZIP application. \* $P < 0.05$ ; error bars, SEMs. (D) The variance-current relationships of the eEPSCs recorded with (red) or without (black) CNQX (0.5  $\mu$ M) for a representative neuron. (E) Voltage dependence of the single channel currents. Open gray circles indicate the individual data collected from nine neurons. (F) Example of peak-scaled NSFA performed on the eEPSCs before and after ZIP application from a neuron in a mouse with nerve injury. (G) ZIP reduced the numbers of active channels selectively in mice with nerve injury. The white and red bars represent the baseline of the sham and nerve injury groups, respectively. The blank blue and solid blue bars indicate the percent change in the number of active channels in sham and nerve injury, respectively, groups 10 min after bath application of ZIP. (H) Digitized photomicrograph showing neurons in laminae II of the spinal cord, which were recorded by stimulating dorsal roots. (I) Example eEPSCs from neurons in lamina II of spinal cord after A $\delta$  or C fiber stimulation. (J) The amplitudes of eEPSCs recorded in nerve injury mice were significantly larger than those in control mice at 1.5 (medium) and 2 times (high) the threshold (low)-stimulating intensity (sham,  $n = 11$ ; nerve injury,  $n = 14$ ; one-way ANOVA,  $F_{1,97} = 10.0$ ,  $P < 0.01$ , Tukey test was used for the multiple comparisons). (K) Pooled data showed that ZIP had similar effects on EPSCs recorded in the spinal cord of nerve injury and sham mice.



III of the ACC 3 or 7 days after nerve injury (24). After obtaining a stable baseline, we bath-applied ZIP (5  $\mu$ M) to block PKM $\zeta$  activity. The evoked EPSCs (eEPSCs) were significantly decreased in six of seven experiments, and total mean responses were reduced to  $83 \pm 6\%$  (mean  $\pm$  SEM) of baseline after 10 min of bath application of ZIP ( $n = 7$ ; Fig. 3, A and C). In contrast, ZIP did not affect the amplitude of eEPSCs recorded in neurons from sham-operated mice ( $n = 6$ ; Fig. 3, B and C).

The ZIP-induced reduction of eEPSCs may be due to a decrease in either the AMPA receptor unitary conductance ( $\gamma$ ) or number of active channels. Peak-scaled nonstationary fluctuation analysis (NSFA) has been developed to distinguish between these parameters (23, 25, 26). As a control, we performed manipulations that should affect either the number of active channels or the single-channel current amplitude but not the single-channel conductance. Similar to previous reports (26), NSFA was able to detect a reduction in the number of active channels induced by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (0.5  $\mu$ M) ( $n = 4$ , paired  $t$  test,  $t_3 = 6.60$ ,  $P <$

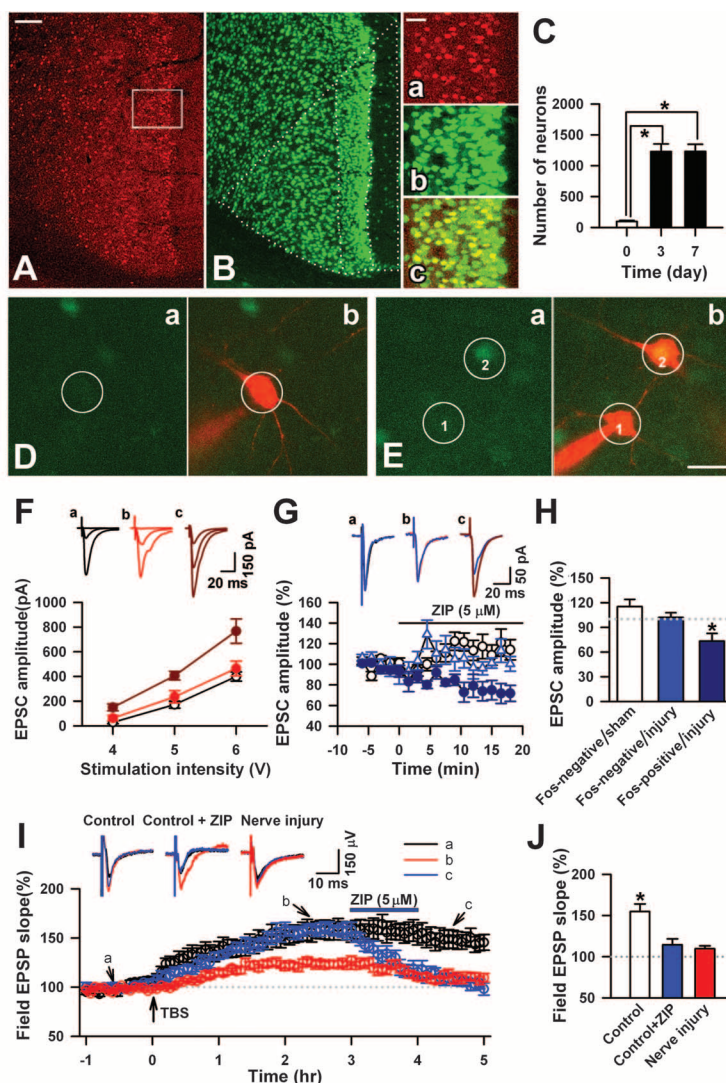
0.05; Fig. 3D) and the change of single-channel current amplitude after an alteration in membrane potential ( $n = 6$  per holding potential; Fig. 3E). NSFA was performed on five of seven neurons where ZIP produced substantial inhibition of the eEPSCs (mean  $76 \pm 5\%$ ); we found that the number of active channels decreased to  $65 \pm 11\%$  ( $n = 5$ , paired  $t$  test,  $t_4 = 6.59$ ,  $P < 0.05$ ) of the baseline (Fig. 3, F and G), whereas  $\gamma$  was unaffected. This effect was not observed in slices obtained from sham-operated mice (Fig. 3G). These results suggest PKM $\zeta$  may exert its effect by increasing the number of AMPA receptors (AMPARs) at synapses in the ACC. A biochemical assay also showed that ZIP infusion reduced postsynaptic GluA1, one component of AMPARs, selectively in the nerve injury group ( $n = 4$  per group, unpaired  $t$  test,  $t_6 = 7.06$ ,  $P < 0.001$ ; fig. S3).

Sensory synaptic transmission in the spinal cord dorsal horn plays important roles in chronic pain and so could be an additional site of action of ZIP, although PKM $\zeta$  and p-PKM $\zeta$  levels in the spinal cord did not change in response to nerve injury. To examine the effects of ZIP on spinal sensory transmission, we recorded eEPSCs from

neurons in lamina II of the spinal cord after dorsal root stimulation. We found that peripheral nerve injury significantly increased the amplitudes of eEPSCs at 1.5 and 2 times of the threshold-stimulating intensity (two-way ANOVA,  $F_{1,97} = 10.00$ ,  $P < 0.01$ ; Fig. 3, H to J). In contrast to the ACC, bath application of ZIP (5  $\mu$ M) to the spinal cord did not distinguish between EPSCs recorded from sham and nerve injury mice (Fig. 3K, two-way ANOVA,  $F_{1,68} = 1.02$ ,  $P > 0.05$ ). In both cases, there was a reduction in sensory transmission (Fig. 3K), which may be due to a constitutive activity of PKM $\zeta$  in spinal sensory neurons. Consistent with these findings, intrathecal injection of ZIP (2 nmol/ $\mu$ l, 5  $\mu$ l per animal) did not produce any analgesic effect ( $n = 4$ , paired  $t$  test,  $t_3 = 0.52$ ,  $P > 0.05$ ).

The ACC is a heterogeneous cortical area, in which not all neurons would be expected to respond to neuropathic pain. We therefore used transgenic mice in which the expression of green fluorescent protein (GFP) is controlled by the promoter of the *c-fos* gene (27, 28), because *c-fos* is known to be activated in sensory neurons after injury (29). In these mice, we found that many

**Fig. 4.** PKM $\zeta$  activity maintains L-LTP in the ACC. (A) Fos-immunopositive cells were found in layers II, III, V, and VI in the ACC of mice 7 days after nerve injury. (B) NeuN immunostaining in the ACC, with dashed line showing the structure of the ACC. The framed area in (A) is magnified in (a). (b) is the magnified image of (B). (c) is the merged result of (a) and (b). Yellow color in (c) represents the Fos/NeuN double-labeled neurons. Scale bar indicates 100  $\mu$ m in (A) and (B) and 30  $\mu$ m in (a) to (c). (C) The total number of Fos-positive neurons were counted in the layers II and III on both sides of the ACC ( $n = 5$  mice in each group). \* $P < 0.001$ ; error bars indicate SEMs. (D) An example of the recorded FosGFP-negative neuron from the ACC of the sham-treated mice. (E) An example showing the double-patched FosGFP-negative (1) and -positive (2) neurons from the ACC of the nerve injury group. (a) images show the expression of FosGFP; (b) images indicate the recorded neurons that were labeled by Alexa fluor 594. Yellow color in (Eb) indicates the overlap of GFP and Alexa fluor 594. Scale bar, 20  $\mu$ m. (F) Pooled data showing the input-output curves of FosGFP-positive neurons from nerve injury mice (dark red) are different from FosGFP-negative neurons of nerve injury (red) and sham-treated (black) mice. Representative eEPSCs recorded from FosGFP-negative neurons (a) of sham-treated or nerve injury (b) mice or FosGFP-positive (c) neurons of nerve injury mice. (G and H) Pooled data showing the effects of ZIP (5  $\mu$ M) on FosGFP-negative [black and (a)] neurons of sham-treated mice and FosGFP-negative [light blue and (b)] and FosGFP-positive [dark blue and (c)] neurons of nerve injury mice. Representative waveforms indicated eEPSCs before (red) and after ZIP (blue) application. \* $P < 0.05$ . (I) L-LTP of the field EPSP induced by the TBS in the ACC can last for at least 5 hours. L-LTP from naïve ( $n = 6$ ) and sham-operated mice ( $n = 5$ ) were similar, and data were pooled together (black). Blocking of the activity of PKM $\zeta$  by bath application of ZIP (5  $\mu$ M) for 1 hour can reverse the synaptic potentiation (blue). L-LTP was occluded in nerve injury mice (red). Sample traces showing the field EPSP recorded at 0.5 hours before TBS conditioning (a) and 2.5 hours (b) and 4.5 hours (c, 0.5 hours after ZIP incubation) after TBS conditioning in the control, ZIP treatment, and nerve injury groups. The blue bar indicates the time scale of ZIP application. (J) Summary data showing ACC L-LTP 4 hours after the induction with ZIP treatment or nerve injury. White, control with TBS conditioning; blue, TBS conditioning with ZIP incubation; red, nerve injury with TBS conditioning. \* $P < 0.05$ .



ACC neurons were activated 3 and 7 days after nerve injury (Fig. 4, A to C). We performed whole-cell patch-clamp recordings from FosGFP-positive pyramidal cells ("green" cells) that were activated by allodynic pain and compared these with nonactivated neighboring neurons and neurons in sham-operated FosGFP mice (Fig. 4, D and E). The amplitudes of eEPSCs stimulated at the same intensity in the FosGFP-positive neurons were significantly greater than those of FosGFP-negative neurons in the ACC of nerve injury and sham-operated mice (two-way ANOVA,  $F_{2,59} = 17.25$ ,  $P < 0.05$ , Fig. 4F). ZIP (5  $\mu$ M) significantly reduced the amplitude of eEPSCs ( $n = 7$ , paired  $t$  test,  $t_6 = 2.85$ ,  $P < 0.05$ ; Fig. 4, G and H) in FosGFP-positive pyramidal cells but had no effect on eEPSCs in either the FosGFP-negative neurons from the same nerve injury group or in neurons from the sham group ( $n = 5$ , nerve injury,  $t_4 = -0.63$ ;  $n = 6$ , sham,  $t_5 = -1.45$ ; paired  $t$  test,  $P > 0.05$ ; Fig. 4, G and H). Furthermore, ZIP had no effect on the eEPSCs recorded on the FosGFP-positive neurons from the primary somatosensory cortex of mice with nerve injury ( $n = 6$ , paired  $t$  test,  $t_5 = -0.55$ ,  $P > 0.05$ ; fig. S2C). In contrast to PKM $\zeta$ , cAMP-dependent protein kinase (PKA) is not required for the maintenance of enhanced EPSCs in the FosGFP-positive neurons, because the PKA inhibitor KT5720 (1  $\mu$ M) did not affect EPSCs ( $n = 5$ , paired  $t$  test,  $t_4 = -0.32$ ,  $P > 0.05$ ; fig. S4). These data suggest that neurons in the ACC that are affected by neuropathic pain have enhanced excitatory synaptic transmission and that this is expressed by a mechanism that is independent of PKA but involves persistent activation of PKM $\zeta$ .

Cortical LTP has been proposed as a cellular model for chronic pain (2). One key function of PKM $\zeta$  is to maintain cortical potentiation at individual synapses (23). To test whether PKM $\zeta$

may be required for maintaining L-LTP in the ACC, we recorded LTP in ACC slices. We induced L-LTP by using a theta burst stimulation (TBS) protocol. The slope of the excitatory postsynaptic potential (EPSP) was significantly greater 3 hours after induction ( $n = 11$ , Fig. 4I). Bath application of ZIP (5  $\mu$ M) 3 hours after induction significantly reduced the potentiation ( $n = 6$ ; Fig. 4, I and J), suggesting that PKM $\zeta$  is required for the maintenance of L-LTP in the ACC. To test whether nerve injury-triggered plasticity shares some common mechanism with L-LTP in the ACC, we performed occlusion experiments. Indeed, synaptic potentiation was significantly reduced in slices of nerve injury mice as compared with those of control mice (unpaired  $t$  test,  $t_{12} = 3.52$ ,  $P < 0.05$ ; Fig. 4, I and J).

We found that PKM $\zeta$  is critical for the maintenance of long-term plasticity in the ACC and contributes to neuropathic pain by modulating excitatory synaptic transmission within this cortical structure. Its role in maintaining synaptic potentiation is a critical part in allodynia resulting from nerve injury. Therefore, PKM $\zeta$  in ACC synapses provides a potential new therapeutic target for the treatment of chronic pain.

#### References and Notes

1. E. R. Kandel, *Science* **294**, 1030 (2001).
2. M. Zhuo, *Trends Neurosci.* **31**, 199 (2008).
3. A. V. Apkarian, M. N. Baliki, P. Y. Geha, *Prog. Neurobiol.* **87**, 81 (2009).
4. M. Costigan, J. Scholz, C. J. Woolf, *Annu. Rev. Neurosci.* **32**, 1 (2009).
5. H. Ikeda *et al.*, *Science* **312**, 1659 (2006).
6. A. Latremoliere, C. J. Woolf, *J. Pain* **10**, 895 (2009).
7. F. Wei, P. Li, M. Zhuo, *J. Neurosci.* **19**, 9346 (1999).
8. T. C. Sacktor, *Prog. Brain Res.* **169**, 27 (2008).
9. E. A. Drier *et al.*, *Nat. Neurosci.* **5**, 316 (2002).
10. P. V. Miguez *et al.*, *Nat. Neurosci.* **13**, 630 (2010).
11. M. U. Naik *et al.*, *J. Comp. Neurol.* **426**, 243 (2000).
12. A. I. Hernandez *et al.*, *J. Biol. Chem.* **278**, 40305 (2003).
13. T. V. Bliss, G. L. Collingridge, S. Laroche, *Science* **313**, 1058 (2006).

14. E. Pastalkova *et al.*, *Science* **313**, 1141 (2006).
15. R. Shema, S. Hazvi, T. C. Sacktor, Y. Dudai, *Learn. Mem.* **16**, 122 (2009).
16. F. Wei *et al.*, *Neuron* **36**, 713 (2002).
17. H. Xu *et al.*, *J. Neurosci.* **28**, 7445 (2008).
18. Materials and methods are available as supporting material on Science Online.
19. K. I. Vadakkan, Y. H. Jia, M. Zhuo, *J. Pain* **6**, 747 (2005).
20. J. Liao, L. J. Wu, M. Zhuo, *J. Neurophysiol.* **94**, 878 (2005).
21. M. T. Kelly, J. F. Cray, T. C. Sacktor, *J. Neurosci.* **27**, 3439 (2007).
22. T. King *et al.*, *Nat. Neurosci.* **12**, 1364 (2009).
23. D. S. Ling, L. S. Benardo, T. C. Sacktor, *Hippocampus* **16**, 443 (2006).
24. L. J. Wu, M. G. Zhao, H. Toyoda, S. W. Ko, M. Zhuo, *J. Neurophysiol.* **94**, 1805 (2005).
25. T. A. Benke, A. Lüthi, J. T. Isaac, G. L. Collingridge, *Nature* **393**, 793 (1998).
26. S. F. Traynelis, R. A. Silver, S. G. Cull-Candy, *Neuron* **11**, 279 (1993).
27. R. L. Clem, T. Celikel, A. L. Barth, *Science* **319**, 101 (2008).
28. A. L. Barth, R. C. Gerkin, K. L. Dean, *J. Neurosci.* **24**, 6466 (2004).
29. S. P. Hunt, A. Pini, G. Evan, *Nature* **328**, 632 (1987).
30. This work was supported by grants from the EJLB-CIHR Michael Smith Chair in Neurosciences and Mental Health, Canada Research Chair, NeuroCanada, and Canadian Institute for Health Research operating grants (CIHR66975 and CIHR84256) (M.Z.). M.Z., B.-K.K., and G.L.C. are also supported by the World-Class University (WCU) program of the Ministry of Education, Science and Technology in Korea through National Research Foundation (R32-10142). X.-Y.L., T.C., and H.W. are supported by postdoctoral fellowships from Fragile X Research Foundation of Canada. B.-K.K. is supported by the National Creative Research Initiative Program of the Korean Ministry of Science and Technology, Korea. H.-G.K. and C.K. are supported by a BK21 fellowship, Korea. G.L.C. receives support from the MRC (UK).

#### Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6009/1400/DC1  
Materials and Methods  
Figs. S1 to S4  
References

3 May 2010; accepted 18 October 2010  
10.1126/science.1191792

## Micro-Optical Sectioning Tomography to Obtain a High-Resolution Atlas of the Mouse Brain

Anan Li,\* Hui Gong,\* Bin Zhang,\* Qingdi Wang, Cheng Yan, Jingpeng Wu, Qian Liu, Shaoqun Zeng, Qingming Luo†

The neuroanatomical architecture is considered to be the basis for understanding brain function and dysfunction. However, existing imaging tools have limitations for brainwide mapping of neural circuits at a mesoscale level. We developed a micro-optical sectioning tomography (MOST) system that can provide micrometer-scale tomography of a centimeter-sized whole mouse brain. Using MOST, we obtained a three-dimensional structural data set of a Golgi-stained whole mouse brain at the neurite level. The morphology and spatial locations of neurons and traces of neurites could be clearly distinguished. We found that neighboring Purkinje cells stick to each other.

One of the most important aims in neuroscience is to obtain an interconnection diagram of the whole brain. In mammals, individual neurons are considered the basic units

of the brain, but the complex functions of the brain depend more on the fine anatomical architecture of a very large number of neurons and their connections (1). Although modern neuroscience

has made great progress in brain studies at both the system and cellular levels, our empirical knowledge of neuroanatomical connectivity remains inadequate, limiting the progress of brain studies (2). Thus, it is necessary to gain new insights into the morphology, localization, and interconnectivity of neural circuits throughout the whole brain at an appropriate resolution. Individual synapses are the finest functional element in circuits, but it is currently not technologically feasible to study the brainwide connectivity of complex vertebrate organisms (e.g., mice) at the synaptic level. In contrast, mesoscale techniques are currently more feasible and applicable for understanding specific neural functions (2).

Light microscopy has remained a key tool for neuroscientists to observe cellular properties at

Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics—Huazhong University of Science and Technology, Wuhan 430074, P. R. China.

\*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: qluo@mail.hust.edu.cn



the mesoscopic level (3). A whole mouse brain is centimeter-sized, however, and is beyond the field of view of modern light microscopy techniques such as confocal and multiphoton microscopy (4, 5). Before microscopic imaging, histological sections are usually prepared for observation of the internal microstructure of large specimens (6–8), but it is difficult to perform serial ultrathin sectioning on large specimens by means of a traditional microtome. In the past decade, several new techniques have been developed to explore the neural circuits of the whole brain (9–14). A common characteristic of the techniques is the use of various kinds of automated sectioning or sectioning-like approaches to increase the detection depth of light microscopes. Some methods start by building section libraries automatically and then imaging these libraries with light or electron microscopy (12, 13). Another method is to perform imaging and sectioning simultaneously without collecting sections, which is a faster, more automated approach (14). Although these techniques have been successfully applied in specific fields, they have not provided a brainwide architecture atlas at the neurite level (3).

We developed an automatic micro-optical sectioning tomography (MOST) instrument to determine the neuronal connectivity of the whole mouse brain at the mesoscopic level. The MOST system is composed of a microtome, light microscope, and image recorder, and it performs imaging and sectioning simultaneously (Fig. 1A and fig. S1).

While working, the microtome slices the specimen into ribbons about 450  $\mu\text{m}$  in width. Once separated from the specimen block, the ribbons are imaged immediately. The light microscope is a reflected bright-field microscope in which the illumination beam is perpendicular to the rake face of the knife and coincides with the imaging beam. As the ribbons are being imaged in motion, a time-delay integration line-scan charge-coupled device (CCD) is used to improve the sensitivity of image recording. All of these design features, especially the optical design, can effectively reduce the system complexity to ensure automation stability and high resolution during time-consuming data acquisition without interruption.

We used MOST to image a whole brain of an adult Kunming (KM) mouse. The brain was from a 5-week-old male with a body weight of 27.57 g and a body length of 173 mm. During specimen preparation (15), staining was performed before embedding; we used a modified Golgi-Cox approach for staining and Spurr resin for embedding. The brain was kept intact throughout specimen preparation. The three anatomical axes of the brain—left-right, dorsal-ventral, and anterior-posterior—corresponded to the three movement directions of the specimen stage, X, Y, and Z. Throughout data acquisition, the section thickness was 1.0  $\mu\text{m}$ , and a water-immersion objective (40 $\times$ , numerical aperture 0.8) was used for imaging. Uninterrupted data acquisition lasted for about 242 hours, covering 15,380 coronal sections.

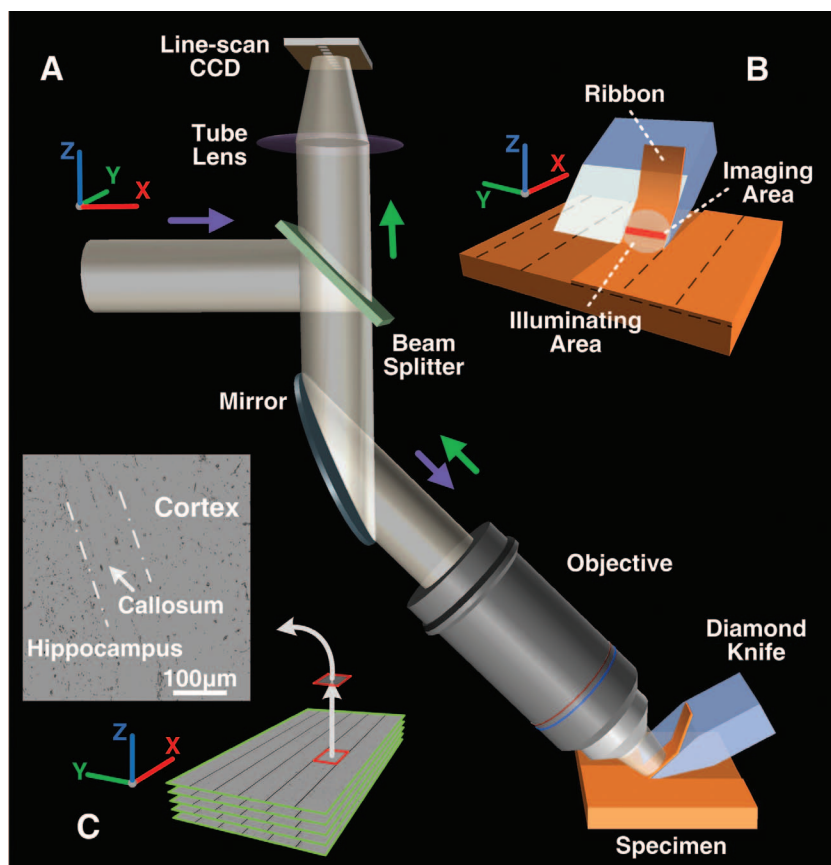
The total amount of uncompressed volume data exceeded 8 terabytes, and the average acquisition rate was about 0.11  $\mu\text{s}$  per voxel, or 0.001  $\text{mm}^3/\text{s}$ .

For optimal visualization of the highly complicated neuronal architecture, the raw mass data should be preprocessed to solve problems with periodic noise and nonuniform brightness. The preprocessed data can then be reconstructed and the volume rendered in three dimensions with the use of the program Amira 5.2.2 to show the whole anatomical sections, small regions of the brain, and individual neurons (15).

Figure 2 shows a series of coronal images reconstructed along the anterior-posterior axis of the entire mouse brain with a spacing of 1 mm. We could also use the original coronal images to reconstruct virtual sagittal images; Fig. 3A shows the reconstruction of such sagittal images. The selected location of the sagittal plane and the abbreviations used in Fig. 3A conform to those in Franklin and Paxinos' atlas (16), and almost all major regions of the brain can be seen. Details of the cerebral cortex, hippocampus, and cerebellum in Fig. 3A are shown in Fig. 3, B to D. The original raw images and additional reconstructions of cross sections are shown in fig. S5 and figs. S7 to S10.

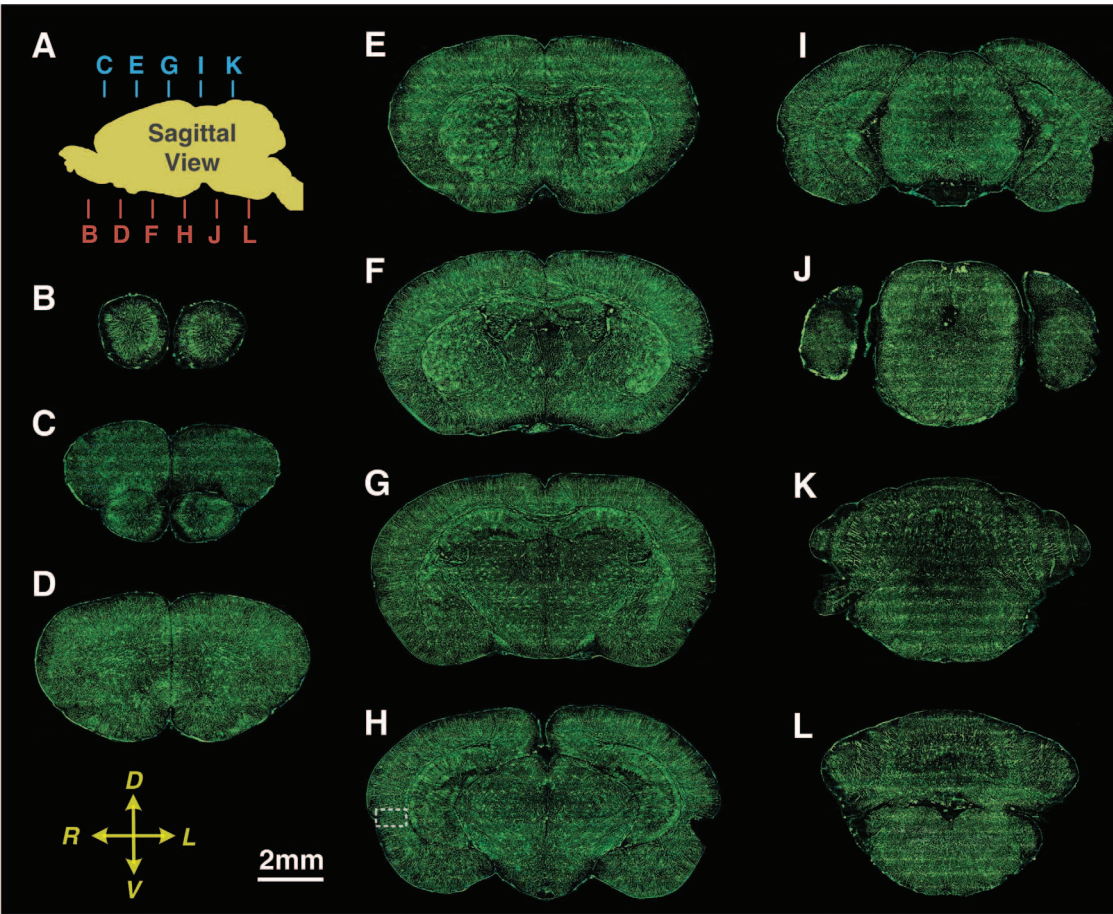
MOST also allows the 3D imaging of neurons and neuronal processes (Fig. 4). Among our sampled reconstructions of the neurons, we found that the neighboring Purkinje cells are close to each other (Fig. 4D and movie S4), which is different

**Fig. 1. (A)** Schematic representation of the MOST system. The specimen is mounted in a chamber, the motion of which is controlled by a series of mechanical translation stages that can move in three directions (the chamber and stages are not shown). Slicing is performed by moving the specimen along the *x* axis to generate ribbons, and each ribbon is simultaneously imaged. The illuminating beam passes through the beam splitter, mirror, and tube lens, the imaging beam collected by the objective is then recorded by a line-scan CCD. **(B)** Schematic representation of slicing. The slicing produces ribbons that glide forward along the knife face. The illuminating and imaging areas are indicated by a circle and a red line, respectively. To expand the detection range, we performed slicing with a lateral and an axial scan (15). **(C)** An image stack acquired using MOST. The stack is composed of many subimages. The subimages aligned along the *x* axis were produced from a ribbon. These image sequences reconstitute the entire cross section of the specimen along the *y* axis. A subimage of the cortex, hippocampus, and corpus callosum is also indicated.

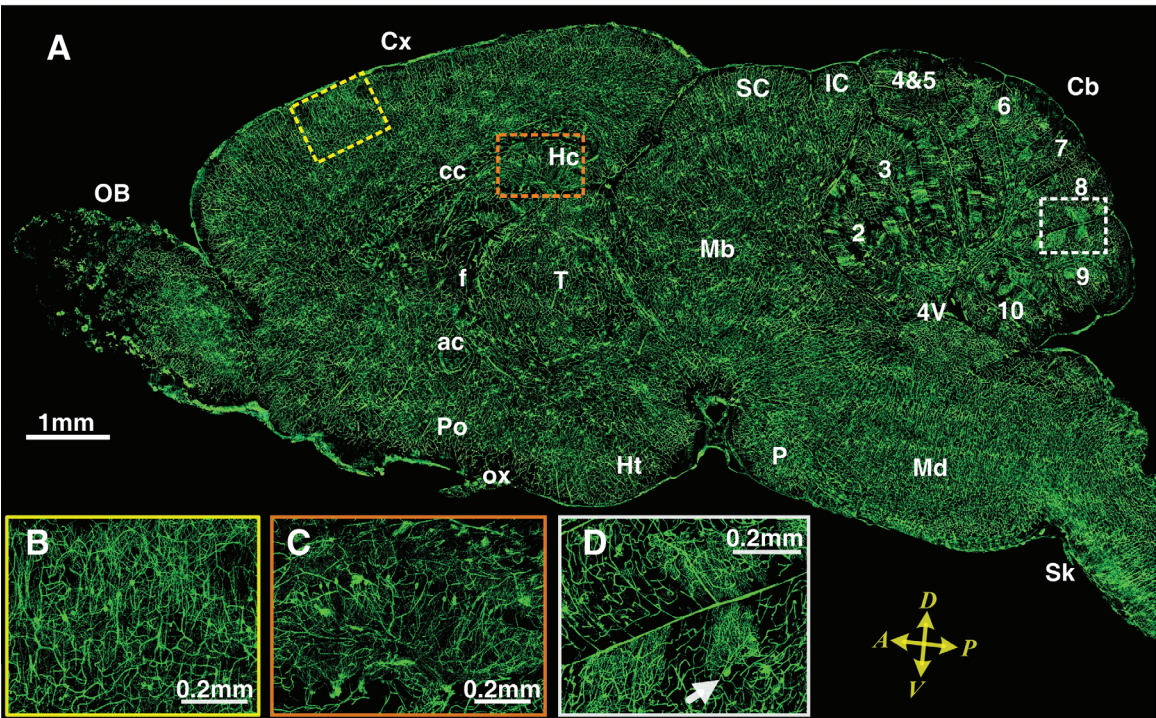




**Fig. 2.** (A) Locations of images shown in (B) to (L), spaced 1 mm apart, from the olfactory bulb to the cerebellum. (B to L) A series of images reconstructed from a stack of 100 coronal sections (total thickness of 0.1 mm). The horizontal stripes, caused by refractive index mismatch between specimen and ambient medium, lead to little information loss. The raw data from MOST are 8-bit grayscale images, and the resulting reconstruction uses a green pseudocolor to enhance their visible effects; dorsal-ventral and left-right axes are indicated at lower left. The dashed box in (H) is described in Fig. 4B.

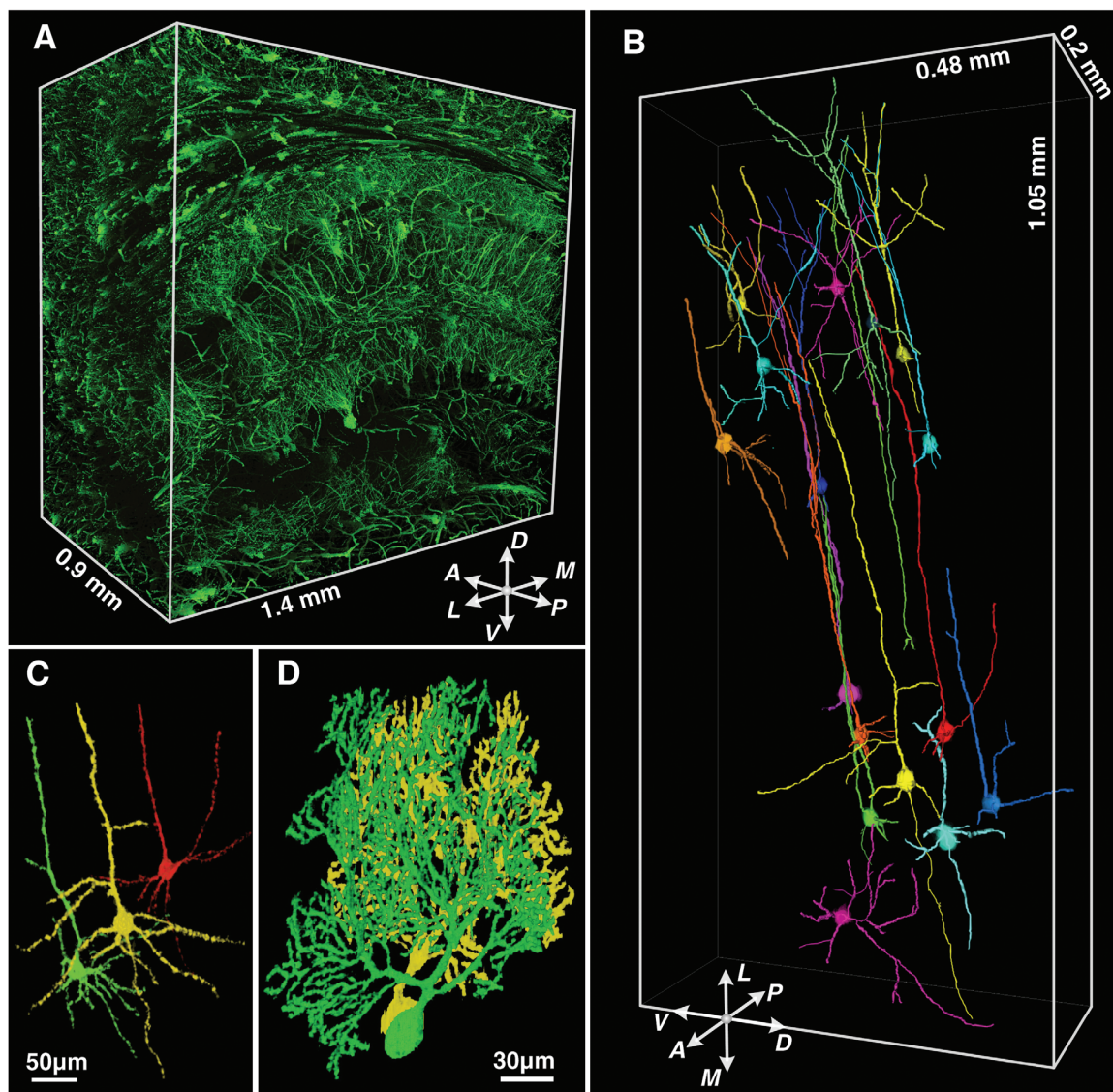


**Fig. 3.** (A) A sagittal image reconstructed from a stack of 100 virtual sagittal sections (total thickness of 0.1 mm). These sections were transformed from the original coronal sections. The sagittal image was located in the right hemisphere about 0.4 mm lateral to the middle. Dorsal-ventral and anterior-posterior axes are indicated. Almost all major regions of the brain can be seen in this image: OB, olfactory bulb; Cx, cerebral cortex; Hc, hippocampus; f, fornix; ac, anterior commissure; T, thalamus; Cb, cerebellum; Mb, midbrain; P, pons; Md, medulla; cc, corpus callosum; SC, superior colliculus; IC, inferior colliculus; Ht, hypothalamus; Po, preoptic area; ox, optic chiasm; 4V, 4th ventricle; arabic numerals 2 to 10, nine lobules of the cerebellum; Sk, spinal cord. The three regions inside the dashed rectangles are the positions of (B), (C), and (D), from left to right. (B to D) The cerebral cortex (B), hippocampus (C), and cerebellum



(D). In the reconstruction of sagittal images, no dislocation was observed along the dorsal-ventral axis; that is, the coronal sections are inherently aligned along the anterior-posterior axis. Arrow in (D) indicates the image described in Fig. 4D.





**Fig. 4.** (A) A large volumetric reconstruction of a partial hippocampus of the mouse brain. The cube volume is 1.4 mm by 1.5 mm by 0.9 mm. The multi-layered structure of the hippocampus and a large number of transverse fibers of the corpus callosum are clearly visible. Dorsal-ventral, anterior-posterior, and left-medial axes are indicated. (B) We traced the neurites of 17 neurons in the ectorhinal cortex, indicated by a dashed box in Fig. 2H. (C)

Three-dimensional reconstruction of three neighboring pyramidal cells in layer 5 of the ectorhinal cortex as in (B). These three cells are distinguished by different colors; other cells, neurites, and blood vessels that densely cover the three cells are not shown. (D) Three-dimensional reconstruction of a pair of neighboring Purkinje cells in the ninth lobule of the cerebellum. The Purkinje cell in green can also be seen in Fig. 3D, indicated by an arrow.

from the corresponding description in *Gray's Anatomy* (17). We also used the program V3D (18) to trace the neurites in the ectorhinal cortex (Fig. 4B and movie S5).

One of the advantages of MOST is that no additional registration is needed because of the accurate spatial positioning of the images (15). Most mechanical slicing methods suffer from serious deformation, which can be overcome when using MOST. Figure 3 shows the smooth contours of the brain tissue surface, and Fig. 4A shows a fine inner structure of the mouse brain; both demonstrate that no registration is needed for the MOST data set. Even at the finer levels, the complex distribution of neurites is shown (e.g., fig. S7). The images are saved with a unified dimension, and their spatial position information is recorded

during data acquisition. If we stack these sub-images in three-dimensional space, a digital whole mouse brain with a voxel size of 0.33  $\mu\text{m}$  by 0.33  $\mu\text{m}$  by 1.0  $\mu\text{m}$  can be reconstructed.

The Golgi method stains a limited number of cells at random in their entirety (15). It is likely that individual neurons or typical structures of the whole brain will be recognized in a complex background and that it will be possible to analyze architectural features with limited computing resources. MOST is capable of fluorescence imaging. Combined with new developments in specimen preparation techniques—for example, the multi-labeled transgenic animal models (19)—the MOST system will help us to obtain a better connectivity map of the entire brain. Such studies will play an important role in functional studies of neural sys-

tems and in understanding and treating various kinds of nervous system diseases.

#### References and Notes

1. E. R. Kandel, in *Principles of Neural Science*, E. R. Kandel, J. H. Schwartz, T. M. Jessell, Eds. (McGraw-Hill, New York, ed. 4, 2000), pp. 19–35.
2. J. W. Bohland *et al.*, *PLOS Comput. Biol.* **5**, e1000334 (2009).
3. B. A. Wilt *et al.*, *Annu. Rev. Neurosci.* **32**, 435 (2009).
4. J. B. Pawley, *Handbook of Biological Confocal Microscopy* (Plenum, New York, ed. 3, 2006).
5. W. Denk, J. H. Strickler, W. W. Webb, *Science* **248**, 73 (1990).
6. M. H. Chin *et al.*, *Physiol. Genomics* **30**, 313 (2007).
7. R. W. Williams, in *Mouse Brain Development*, A. M. Goffinet, P. Rakic, Eds. (Springer-Verlag, New York, 2000), pp. 21–50.
8. E. S. Lein *et al.*, *Nature* **445**, 168 (2007).
9. P. S. Tsai *et al.*, *Neuron* **39**, 27 (2003).
10. J. Huisken, J. Swoger, F. Del Bene, J. Wittbrodt, E. H. K. Stelzer, *Science* **305**, 1007 (2004).

11. H. U. Dodt *et al.*, *Nat. Methods* **4**, 331 (2007).
12. K. D. Micheva, S. J. Smith, *Neuron* **55**, 25 (2007).
13. K. J. Hayworth, N. Kasthuri, R. Schalek, J. W. Lichtman, *Microsc. Microanal.* **12** (S02), 86 (2006).
14. D. Mayerich, L. Abbott, B. McCormick, *J. Microsc.* **231**, 134 (2008).
15. See supporting material on Science Online.
16. K. B. J. Franklin, G. Paxinos, *The Mouse Brain in Stereotaxic Coordinates* (Academic Press, San Diego, CA, ed. 3, 2007).
17. S. Standing, *Gray's Anatomy: The Anatomical Basis of Clinical Practice* (Churchill Livingstone, London, ed. 39, 2004).
18. H. C. Peng, Z. C. Ruan, F. H. Long, J. H. Simpson, E. W. Myers, *Nat. Biotechnol.* **28**, 348 (2010).
19. G. Feng *et al.*, *Neuron* **28**, 41 (2000).
20. We thank P. Wu, J. B. Wu, and L. Wu for their early work in MOST; L. Fu, T. H. Xu, and W. Zhou for helpful discussions; Z. Dong and Z. Feng for assistance in experiments; J. Zhou for the nanowire sample; and Diatome AG, Switzerland, for the diamond knife. Supported by the National Natural Science Foundation of China (grants 30727002, 60025514, 30700214, and 30925013) and the Program for Changjiang Scholars and Innovative Research Team in University.

### Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1191776/DC1  
Materials and Methods  
SOM Text  
Figs. S1 to S10  
Movies S1 to S5  
References

3 May 2010; accepted 23 September 2010  
Published online 4 November 2010;  
10.1126/science.1191776

# Paradoxical False Memory for Objects After Brain Damage

Stephanie M. McTighe,<sup>1,2</sup> Rosemary A. Cowell,<sup>3</sup> Boyer D. Winters,<sup>4</sup>  
Timothy J. Bussey,<sup>1,2</sup> Lisa M. Saksida<sup>1,2\*</sup>

Poor memory after brain damage is usually considered to be a result of information being lost or rendered inaccessible. It is assumed that such memory impairment must be due to the incorrect interpretation of previously encountered information as being novel. In object recognition memory experiments with rats, we found that memory impairment can take the opposite form: a tendency to treat novel experiences as familiar. This impairment could be rescued with the use of a visual-restriction procedure that reduces interference. Such a pattern of data can be explained in terms of a recent representational-hierarchical view of cognition.

**A**lthough individuals with some types of brain damage—for example, to structures in the medial temporal lobe—are able to remember new information immediately after it is learned, their recall after even a very short in-

terval can be greatly compromised or nonexistent (1). It would seem to follow that the experiences of such individuals must be lost rapidly or are inaccessible, such that when a novel object is encountered and later reexperienced, it is as if the object had never before been seen. We explored this assumption with an animal model of object recognition memory that has been widely used as a model of memory impairment (2).

The standard object recognition procedure involves exposure to a study object, followed (after a delay) by a test phase in which the study object is again presented, along with a perceptually distinct novel object. The participant's task is to dis-

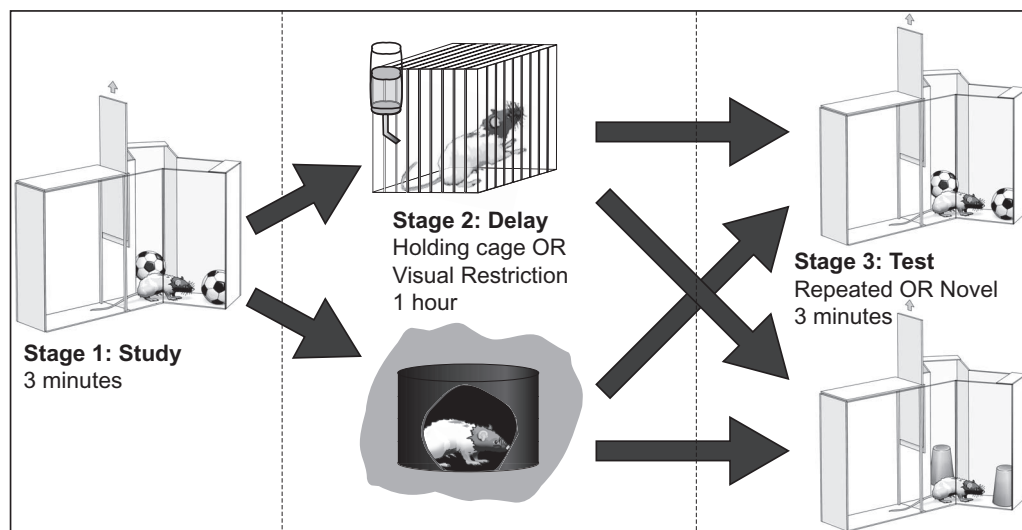
tinguish the novel from the repeated object. Animals and human subjects with damage to the perirhinal cortex are impaired on this task when a sufficiently long delay is interposed between study and test (2–4); indeed, much work has established that damage to the perirhinal cortex alone is sufficient to cause severe impairments in object recognition (2, 5–8). When the standard task procedure is used, the requirement that two items be simultaneously compared precludes determination of whether the novel item is viewed as familiar, or vice versa, because the presence of one item affects how much the other is explored and how it is evaluated.

We therefore devised a method of assessing object recognition that decouples the subjects' evaluation of novel and repeated objects (Fig. 1) (9). Consistent with previous studies, intact rats explored in the novel-object condition more than in the repeated-object condition, thus showing intact memory for the repeated object. Also consistent with previous studies, rats with perirhinal cortex damage explored novel and repeated objects equally, indicating an inability to distinguish between them (2). Paradoxically, however, this inability to distinguish novel from repeated objects was characterized not by an increase in exploration of the repeated object—which would have indicated that they judged the repeated object as novel—but by a decrease in their exploration of the novel object, indicating that they

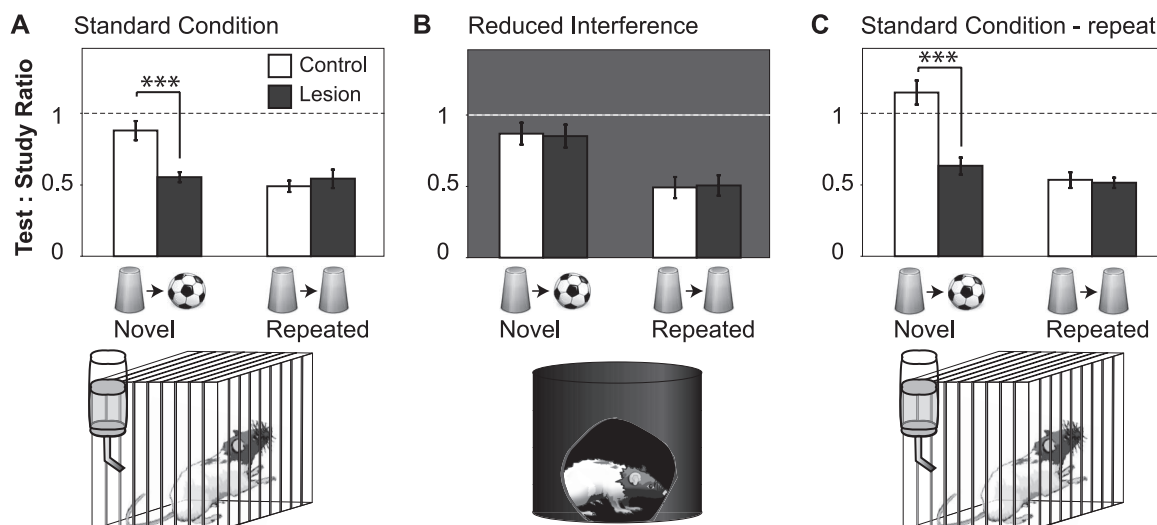
<sup>1</sup>Department of Experimental Psychology, University of Cambridge, Cambridge CB2 3EB, UK. <sup>2</sup>MRC and Wellcome Trust Behavioural and Clinical Neuroscience Institute, University of Cambridge, Cambridge CB2 3EB, UK. <sup>3</sup>Department of Psychology, University of California, San Diego, La Jolla, CA 92093, USA. <sup>4</sup>Department of Psychology, University of Guelph, Guelph, Ontario N1G 2W1, Canada.

\*To whom correspondence should be addressed. E-mail: lms42@cam.ac.uk

**Fig. 1.** A modified version of the spontaneous object recognition task, in which exploration of the repeated object is decoupled from exploration of the novel object. In the novel-object condition, an animal received a study exposure to two copies of object A for 3 min. Then the animal was put into an individual holding cage (standard condition) or a visually restricted environment (reduced-interference condition) for a delay of 1 hour. After the delay, the animal received a test exposure of 3 min to two copies of a novel object, object B. In the repeated-object condition, the animal received a study exposure of 3 min to two copies of object A. Then, as before, the animal was put into an individual holding cage or a visually restricted environment for a delay of 1 hour. After the delay, the animal received a test exposure of 3 min to two copies of the familiar object, object A.







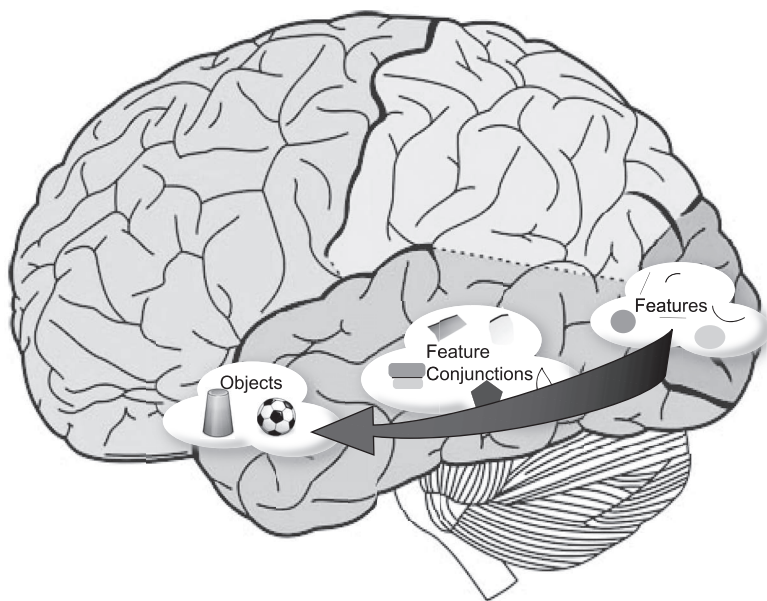
**Fig. 2.** Performance of rats with perirhinal cortex damage on the object recognition task. **(A)** Standard condition, with normal interference-filled delay. Repeated-measures analysis of variance (ANOVA) showed a significant effect of condition ( $F_{1,19} = 15.38$ ,  $P < 0.001$ ), an effect of lesion ( $F_{1,19} = 5.39$ ,  $P < 0.05$ ), and a significant interaction ( $F_{1,19} = 13.58$ ,  $P < 0.005$ ). Post hoc  $t$  tests using Bonferroni correction showed a significant difference in the novel condition ( $t_{19} = 3.88$ ,  $P < 0.001$ ) and no difference in the repeated-object condition ( $t_{19} = 0.76$ ,  $P > 0.05$ ), indicating that lesioned animals treated the novel object as though it were familiar. **(B)** Reduced-interference condition in which animals were placed into a visually restricted environment during the delay. Repeated-measures

ANOVA showed a significant effect of condition ( $F_{1,19} = 18.33$ ,  $P < 0.001$ ), no effect of lesion ( $F < 1$ ), and no interaction ( $F < 1$ ), indicating that both groups discriminated the objects after visual restriction. **(C)** Repetition of the standard condition. Repeated-measures ANOVA showed a significant effect of condition ( $F_{1,18} = 33.97$ ,  $P < 0.001$ ), a significant effect of lesion ( $F_{1,18} = 14.59$ ,  $P < 0.005$ ), and a significant interaction ( $F_{1,18} = 16.17$ ,  $P < 0.005$ ). Post hoc  $t$  tests using Bonferroni correction showed a significant difference in the novel condition ( $t_{18} = 4.62$ ,  $P < 0.001$ ) and no difference in the repeated condition ( $t_{18} = 0.33$ ,  $P > 0.05$ ), showing that the impairment returned when normal levels of interference were reinstated. \*\*\* $P < 0.001$ .

treated the novel object as though it were familiar (Fig. 2A) (9).

Our previous work has suggested that impairments in this task may be a result of interference during the delay (10) and has shown that the explicit addition of interfering objects during the delay can impair memory in animals with perirhinal cortex damage (11). Accordingly, we reduced interference during the delay by putting the animals into a dark environment between the study and test phases (9, 12). This treatment completely rescued the performance of the lesioned animals (Fig. 2B) (9). To ensure that the rescue of the impairment was not due to brain reorganization or recovery, we retested the animals under the original experimental conditions, and the impairment returned (Fig. 2C).

The finding that the object recognition memory impairment in this model was characterized by animals judging novel objects as familiar seems counterintuitive. It is difficult to reconcile with the assumption that all information presented during the study phase is lost or inaccessible, because this assumption would suggest that animals should judge repeated objects as novel. An alternative view (13–15), however, suggests that memory loss after brain damage may be better understood, not in terms of loss of a system dedicated to a specific type of memory—for example, long- versus short-term memory, or memory processes such as encoding, storage/consolidation, or retrieval (16)—but in terms of the stimulus representations that the different regions contain (Fig. 3) (17). For visual



**Fig. 3.** The representational-hierarchical view. Representations of visual stimulus features are organized hierarchically in increasingly complex conjunctions as information flows from posterior to anterior regions of the ventral visual stream (18, 19). The representation of a particular object is distributed throughout the system, but it is represented differently at different points: as features early in the ventral visual stream, as conjunctions of features in intermediate regions in the ventral visual stream, and as highly complex conjunctions of features—shown here at the level of object wholes—in the perirhinal cortex. The traditional multiple memory systems view suggests that structures within the medial temporal lobe subserve exclusively mnemonic function, whereas structures in the ventral visual stream are important for perceptual functions. In contrast, the representational-hierarchical view suggests that stimulus representations throughout the ventral-visual-perirhinal-hippocampal stream are useful for any cognitive function that requires them.

input, stimulus representations are organized hierarchically, with conjunctive representations of relatively complex stimuli in anterior brain regions, and with representations of relatively simple features in more posterior regions (18, 19). We have suggested that this representational hierarchy may extend into structures within the medial temporal lobe, and that it is the highly complex conjunctive representations of stimuli that are maintained in regions such as the perirhinal cortex (13–15). According to this view, individuals with damage to such regions should be impaired not just on a particular type of memory function, but also on any cognitive function that requires the complex stimulus representations that are damaged.

This representational-hierarchical view accounts for the seemingly paradoxical findings of the present study. Indeed, the pattern of results is a central prediction of the model, as illustrated by simulations using a computational model (9, 10). In these simulations, it is assumed that during the delay between study and test, the subject will be exposed to other, non-experimental visual material that occurs naturally in the environment. This material will almost certainly share some features in common with the novel object. This can lead to interference, because as a result of this exposure, features in the novel object will now be familiar. However, because it is very unlikely that the specific complex object presented during the test phase will have been experienced by chance during the delay, the unique, object-level representations in anterior regions of the system will not be familiar and therefore can protect the system from this interference. If these conjunctive representations of complex stimuli are damaged, however, then the subject will have to rely on the simpler, feature-based memory that is highly susceptible to interference. From this theoretical framework, we obtain the perhaps counterintuitive prediction that brain damage can produce impairments in visual recognition memory tasks not because the repeated object looks novel, but because the novel object looks familiar (fig. S3A). The clear corollary of this prediction is that reducing interference in the delay should improve performance in memory-impaired subjects (fig. S3B), which is what we found (Fig. 2B).

These simulations and the current results suggest that object recognition memory impairments may not be due to damage to a dedicated memory system from which all information presented in the study phase is lost or inaccessible. Instead, brain damage that leads to such impairments compromises only a very specific type of complex stimulus representation. Other, simpler feature-level representations of the repeated item remain. Because these remaining, relatively simple stimulus features tend to be repeated across objects and situations, their representations do not provide a unique signal of prior occurrence of an object. As a result, the disruption to the encoding of complex stimulus representations has the knock-on effect of making the system very susceptible to interference. The fact that only a portion of

the representation of an object is affected by the lesion is indicated by the lack of impairment when lesioned animals spent the delay in a visually restricted environment. This intact performance must be supported by representations outside the perirhinal cortex, as predicted by the representational-hierarchical view (9). This shows that object recognition impairments after perirhinal cortex damage cannot be understood in the usual way—that is, as the result of damage to all or part of a dedicated memory system. The present results also add to a growing body of evidence suggesting that interference may be an important factor in memory impairments after brain damage (12, 20–26).

The combination of perirhinal lesions and object recognition used in our study models the disturbances in the recognition of objects and people that are critical components of amnesia. It does not, however, model all forms of memory impairment; for example, it is not a complete model of the dense, global amnesic syndrome observed in people with extensive damage to medial temporal lobe structures including the hippocampus. Nonetheless, these findings raise the possibility that the false memory reported here may be a more general phenomenon. If this turns out to be the case, we may need to reevaluate our current conceptions of memory impairment and amnesia.

#### References and Notes

1. A. D. Baddeley, E. K. Warrington, *J. Verbal Learn. Verbal Behav.* **9**, 176 (1970).
2. B. D. Winters, L. M. Saksida, T. J. Bussey, *Neuropsychologia* **48**, 2251 (2010).
3. P. Alvarez, S. Zola-Morgan, L. R. Squire, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 5637 (1994).
4. D. G. Mumby, J. P. Pinel, *Behav. Neurosci.* **108**, 11 (1994).
5. T. J. Bussey, J. L. Muir, J. P. Aggleton, *J. Neurosci.* **19**, 495 (1999).

6. A. Ennaceur, N. Neave, J. P. Aggleton, *Behav. Brain Res.* **80**, 9 (1996).
7. R. E. Clark, L. R. Squire, *Neuropsychologia* **48**, 2234 (2010).
8. D. G. Mumby, *Behav. Brain Res.* **127**, 159 (2001).
9. See supporting material on Science Online.
10. R. A. Cowell, T. J. Bussey, L. M. Saksida, *J. Neurosci.* **26**, 12186 (2006).
11. S. J. Bartko, R. A. Cowell, B. D. Winters, T. J. Bussey, L. M. Saksida, *Neuropsychologia* **48**, 2987 (2010).
12. N. Cowan, N. Beschin, S. Della Sala, *Brain* **127**, 825 (2004).
13. T. J. Bussey, L. M. Saksida, *Hippocampus* **17**, 898 (2007).
14. E. A. Murray, T. J. Bussey, L. M. Saksida, *Annu. Rev. Neurosci.* **30**, 99 (2007).
15. L. M. Saksida, T. J. Bussey, *Neuropsychologia* **48**, 2370 (2010).
16. B. D. Winters, T. J. Bussey, *J. Neurosci.* **25**, 52 (2005).
17. L. M. Saksida, *Science* **325**, 40 (2009).
18. D. J. Felleman, D. C. Van Essen, *Cereb. Cortex* **1**, 1 (1991).
19. L. G. Ungerleider, M. Mishkin, in *Analysis of Visual Behavior*, D. J. Ingle, M. A. Goodale, R. J. W. Mansfield, Eds. (MIT Press, Cambridge, MA, 1982), pp. 549–586.
20. E. K. Warrington, L. Weiskrantz, *Nature* **228**, 628 (1970).
21. J. T. Wixted, *Annu. Rev. Psychol.* **55**, 235 (2004).
22. S. Della Sala, N. Cowan, N. Beschin, M. Perini, *Memory* **13**, 435 (2005).
23. M. Dewar, Y. F. Garcia, N. Cowan, S. Della Sala, *Neuropsychology* **23**, 627 (2009).
24. M. T. Dewar, N. Cowan, S. D. Sala, *Cortex* **43**, 616 (2007).
25. E. K. Warrington, L. Weiskrantz, *Neuropsychologia* **12**, 419 (1974).
26. E. K. Warrington, L. Weiskrantz, *Neuropsychologia* **16**, 169 (1978).
27. Supported by a grant from the BBSRC (L.M.S., T.J.B., B.D.W.), and a 3-year MRC-funded Ph.D. studentship at the University of Cambridge, MRC/Wellcome Trust Behavioral and Clinical Neuroscience Institute (S.M.M.).

#### Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6009/1408/DC1  
Materials and Methods  
Figs. S1 to S3  
Tables S1 to S13  
References

7 July 2010; accepted 19 October 2010  
10.1126/science.1194780

## Frequent Mutation of *BAP1* in Metastasizing Uveal Melanomas

J. William Harbour,<sup>1,3\*</sup> Michael D. Onken,<sup>1</sup> Elisha D. O. Roberson,<sup>2</sup> Shenghui Duan,<sup>2</sup> Li Cao,<sup>2</sup> Lori A. Worley,<sup>1</sup> M. Laurin Council,<sup>2</sup> Katie A. Matatall,<sup>1</sup> Cynthia Helms,<sup>2</sup> Anne M. Bowcock<sup>2,3\*</sup>

Metastasis is a defining feature of malignant tumors and is the most common cause of cancer-related death, yet the genetics of metastasis are poorly understood. We used exome capture coupled with massively parallel sequencing to search for metastasis-related mutations in highly metastatic uveal melanomas of the eye. Inactivating somatic mutations were identified in the gene encoding BRCA1-associated protein 1 (*BAP1*) on chromosome 3p21.1 in 26 of 31 (84%) metastasizing tumors, including 15 mutations causing premature protein termination and 5 affecting its ubiquitin carboxyl terminal hydrolase domain. One tumor harbored a frameshift mutation that was germline in origin, thus representing a susceptibility allele. These findings implicate loss of *BAP1* in uveal melanoma metastasis and suggest that the *BAP1* pathway may be a valuable therapeutic target.

**U**veal melanoma (UM) is the most common primary cancer of the eye and has a strong propensity for fatal metastasis (1). UMs are divided into class 1 (low metastatic risk) and class 2 (high metastatic risk) based on a validated multi-

gene clinical prognostic assay included in the TNM classification system (2, 3). However, the genetic basis of metastasis remains unclear. Oncogenic mutations in the  $G\alpha_q$  stimulatory subunit *GNAQ* are common in UM (4), but these mutations occur



early in tumorigenesis and are not correlated with molecular class or metastasis (5, 6). On the other hand, class 2 tumors are strongly associated with monosomy 3 (7), suggesting that loss of one copy of chromosome 3 may unmask a mutant gene on the remaining copy that promotes metastasis.

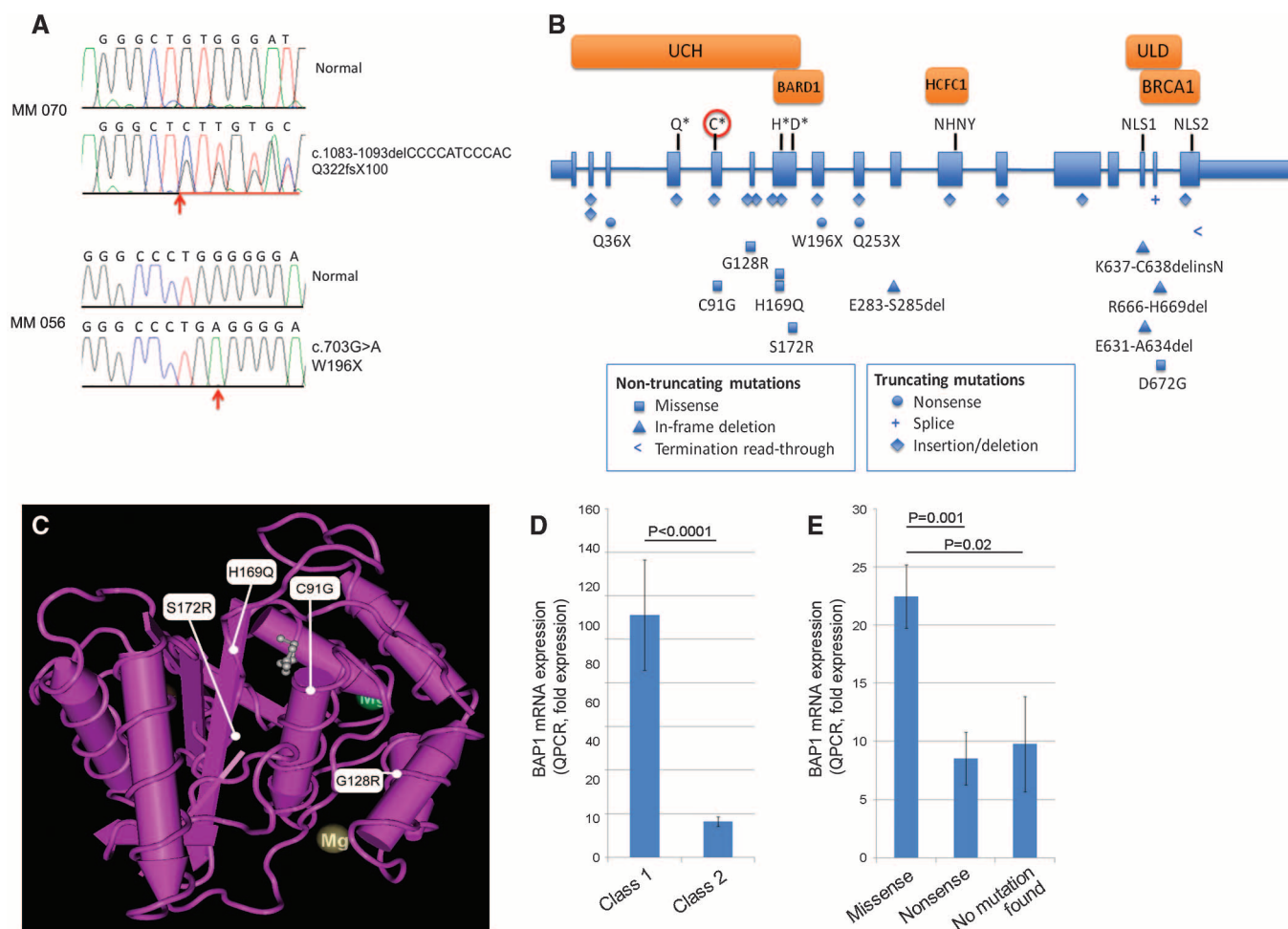
<sup>1</sup>Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, MO 63110, USA. <sup>2</sup>Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110, USA. <sup>3</sup>Siteman Cancer Center, Washington University School of Medicine, St. Louis, MO 63110, USA.

\*To whom correspondence should be addressed. E-mail: bowcock@genetics.wustl.edu (A.M.B.); harbour@vision.wustl.edu (J.W.H.)

Using exome capture followed by massively parallel sequencing (8–10), we analyzed two class 2 tumors that were monosomic for chromosome 3 (MM 56 and MM 70) and matching normal DNA from peripheral blood lymphocytes. Both tumors contained inactivating mutations in *BAP1*, located at chromosome 3p21.1 (Fig. 1A) (10). MM 56 contained a C/G to T/A transition that created a premature termination codon (p.W196X). MM 70 contained a deletion of 11 base pairs in exon 11, leading to a frameshift and premature termination of the BAP1 protein (p.Q322fsX100). The matched normal DNA samples did not contain these mutations, indicating that they were likely to be somatic in origin. No gene on chromosome 3 other

than *BAP1* contained deleterious somatic mutations that were present in both tumors (table S3).

*BAP1* encodes a nuclear ubiquitin carboxy-terminal hydrolase (UCH), one of several classes of deubiquitinating enzymes (11). In addition to the UCH catalytic domain, BAP1 contains a UCH37-like domain (ULD) (12), binding domains for BRCA1 and BARD1, which form a tumor suppressor heterodimeric complex (13), and a binding domain for HCFC1, which interacts with histone-modifying complexes during cell division (12, 14, 15). BAP1 also interacts with ASXL1 to form the Polycomb group repressive deubiquitinase complex (PR-DUB), which is involved in stem cell pluripotency and other developmental processes



**Fig. 1.** Inactivating mutations in *BAP1* occur frequently in uveal melanomas. (A) Sanger sequence traces of MM 056 and MM 070 at the sites of the mutations. Location of mutated base in MM 056 and the start of the deletion of MM 070 are indicated (arrows). The noncoding *BAP1* strand is shown for MM 070. (B) Map of *BAP1* gene and location of *BAP1* mutations. *BAP1* contains 17 exons (shaded boxes) that encode a 728–amino acid protein. Introns are not to scale. Mutations are shown below the gene figure as indicated. The UCH domain [amino acids (aa) 1 to 188] and UCH37-like domain (ULD) (aa 635 to 693) are indicated (12, 13). The critical Q, C, H, and D residues of the active site (Gln<sup>85</sup>, Cys<sup>91</sup>, His<sup>169</sup>, and Asp<sup>184</sup>) are indicated with asterisks. The catalytic cysteine is indicated with a circle. Also shown are the NHNY consensus sequence for interaction with HCFC1 (aa 363 to 365, exon 11), nuclear localization signals (NLS) at aa 656 to 661 (exon 15) and aa 717 to 722 (exon 17), the BARD1 binding domain within the region bounded by aa 182 to 240 (13), and the BRCA1 binding domain within aa

598 to 729 (11). (C) Location of *BAP1* missense mutations in the UCH domain aligned to the crystal structure of *UCH-L3* (21). Three-dimensional structure of *UCH-L3* was visualized with MMDB software (22). The small molecule near C91G, H169Q, and S172R represents a suicide inhibitor, illustrating the critical location of these mutations for catalytic activity. (D) *BAP1* mRNA levels measured by quantitative RT-PCR in 9 nonmetastasizing class 1 UMs and 28 metastasizing class 2 UMs. (E) Relationship between *BAP1* mRNA levels (measured by quantitative RT-PCR) and type of *BAP1* mutation in 9 UMs with nonsense and other truncating mutations, 10 UMs with missense mutations (together with small in-frame deletions, splice acceptor, and stop codon read-through mutations), and 4 class 2 UMs in which no *BAP1* mutations were detected. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

(16, 17). BAP1 exhibits tumor suppressor activity in cancer cells (11, 13), and *BAP1* mutations have been reported in a small number of breast and lung cancer samples (11, 18).

To further investigate *BAP1*, genomic DNA from 29 additional class 2 UMs and 26 class 1 UMs were subjected to Sanger resequencing of all *BAP1* exons. Altogether, *BAP1* mutations were identified in 26 of 31 (84%) class 2 tumors, including 13 out-of-frame deletions and two nonsense mutations leading to premature protein termination, six missense mutations, four in-frame deletions, and one mutation predicted to produce an abnormally extended BAP1 polypeptide (Fig. 1, A to C) (10). Three of the missense mutations affected catalytic residues of the UCH active site (C91 and H169), two occurred elsewhere in the UCH domain, and one affected the ULD (Fig. 1, B and C). All *BAP1* missense mutations and in-frame deletions affected phylogenetically conserved amino acids (fig. S1). Only one of 26 class 1 tumors contained a *BAP1* mutation (NB101) (10). This case may represent a transition state in which the tumor has sustained a *BAP1* mutation but has not yet converted to class 2, suggesting that *BAP1* mutations may precede the emergence of the class 2 signature. Somatic *BAP1* mutations were also detected in two of three metastatic tumors (10).

One copy of chromosome 3 was missing in all 17 *BAP1*-mutant class 2 tumors for which

cytogenetic data were available, consistent with chromosome 3 loss uncovering recessive *BAP1* mutations (10). Normal DNA from 20 patients with *BAP1*-mutant class 2 primary tumors and the two with metastatic tumors was available and did not contain a *BAP1* mutation, indicating that the mutations were somatic in origin. However, we detected one germline mutation (p.E402fsX2; c.1318-1319insA) in the patient with tumor MM 087 (table S2), suggesting that germline alterations in *BAP1* can predispose to UM. *GNAQ* mutation status was available in 15 cases. *GNAQ* mutations were present in 4 of 9 *BAP1* mutant tumors and 3 of 6 *BAP1* wild-type tumors, indicating that there was no correlation between *GNAQ* and *BAP1* mutation status.

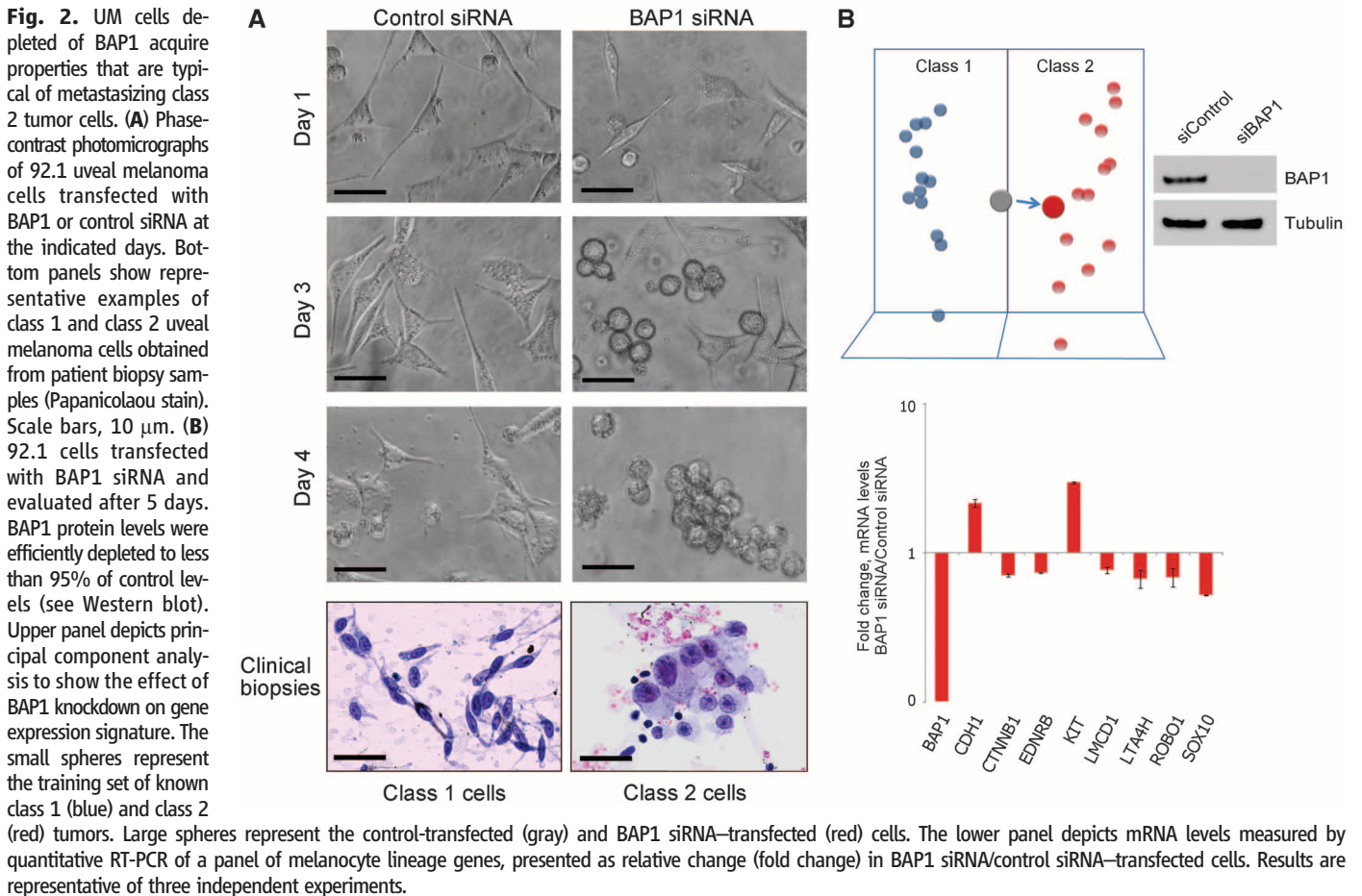
Quantitative reverse transcription polymerase chain reaction (RT-PCR) showed that *BAP1* mRNA levels were significantly lower in class 2 tumors compared with class 1 tumors ( $P < 0.0001$ ) (Fig. 1D). Truncating mutations were associated with significantly lower mRNA levels than missense mutations ( $P = 0.001$ ) (Fig. 1E), consistent with nonsense-mediated mRNA decay in the former group. Class 2 tumors in which *BAP1* mutations were not identified expressed very low levels of *BAP1* mRNA (Fig. 1E).

To determine whether the low BAP1 mRNA levels in class 2 tumors without detectable BAP1 mutations may be explained by DNA methyla-

tion, we performed a preliminary analysis of DNA methylation of BAP1. This did not reveal a convincing difference between class 1 and class 2 tumors (10). However, analysis of the BAP1 promoter was limited by an unusually complex CpG island that will require further work to resolve. Thus, we cannot rule out a role for methylation in class 2 tumors in which BAP1 mutations were not found. However, with almost 85% of class 2 tumors harboring mutations, we do not expect that methylation will be a major mechanism of BAP1 inactivation. An alternative explanation is that these tumors may contain very large deletions of the *BAP1* locus not detectable by our sequencing method.

Immunofluorescence revealed abundant nuclear BAP1 protein in two class 1 tumors but virtually none in four *BAP1* mutant class 2 tumors (fig. S3). This was expected for the two tumors with mutations expected to cause premature protein terminations (MM 091 and MM 100), but it was surprising for the two tumors with missense mutations (MM 071 and MM 135) and suggests that these mutations lead to protein instability.

RNA interference (RNAi)-mediated knock down of BAP1 in 92.1 UM cells, which did not harbor a detectable *BAP1* mutation, recapitulated many characteristics of the dedifferentiated class 2 UM phenotype (19). Cells transfected with control small interfering RNA (siRNA) exhibited typical melanocytic morphology, including dendritic





projections and cytoplasmic melanosomes (Fig. 2A), whereas cells transfected with BAP1 siRNA lost these features, developed a rounded epithelioid morphology, and grew as multicellular non-adherent spheroids, strikingly similar to the features of class 2 clinical biopsy samples (Fig. 2A). Microarray gene expression profiling of 92.1 UM cells transfected with control versus BAP1 siRNA showed that most of the top genes that discriminate between class 1 and class 2 tumors shifted in the class 2 direction in BAP1-depleted cells compared with control cells (fig. S4). Similarly, depletion of BAP1 shifted the gene expression profile of the multi-gene clinical prognostic assay toward the class 2 signature (Fig. 2B). BAP1 depletion caused a reduction in mRNA levels of neural crest migration genes (*ROBO1*), melanocyte differentiation genes (*CTNBN1*, *EDNRB*, and *SOX10*), and other genes that are down-regulated in class 2 tumors (*LMCD1* and *LTA4H*) (19). In contrast, BAP1 depletion caused an increase in mRNA levels of *CDH1* and the proto-oncogene *KIT*, which are highly expressed in class 2 tumors (20). Similar results were seen in other UM cell lines and with an independent BAP1 siRNA (10).

GNAQ mutations occur early in UM and are not sufficient for malignant transformation (4), but they may create a dependency of the tumor cells on constitutive GNAQ activity. In contrast, BAP1 mutations occur later in UM progression and coincide with the onset of metastatic behav-

ior. Thus, simultaneous targeting of both genetic alterations might have synergistic therapeutic effects. One potential strategy to counteract the effects of BAP1 mutation would be to inhibit the RING1 ubiquitinating activity that normally opposes the deubiquitinating activity of BAP1 (16). Our findings strongly implicate mutational inactivation of BAP1 as a key event in the acquisition of metastatic competence in UM, and they expand the role of BAP1 and other deubiquitinating enzymes as potential therapeutic targets in cancer.

#### References and Notes

1. S. Landreville, O. A. Agapova, J. W. Harbour, *Future Oncol.* **4**, 629 (2008).
2. M. D. Onken, L. A. Worley, M. D. Tuscan, J. W. Harbour, *J. Mol. Diagn.* **12**, 461 (2010).
3. P. T. Finger, 7th Edition, AJCC-UICC Ophthalmic Oncology Task Force, *Arch. Pathol. Lab. Med.* **133**, 1197 (2009).
4. C. D. Van Raamsdonk et al., *Nature* **457**, 599 (2009).
5. M. D. Onken et al., *Invest. Ophthalmol. Vis. Sci.* **49**, 5230 (2008).
6. J. Bauer et al., *Br. J. Cancer* **101**, 813 (2009).
7. L. A. Worley et al., *Clin. Cancer Res.* **13**, 1466 (2007).
8. S. Bashiardes et al., *Nat. Methods* **2**, 63 (2005).
9. S. B. Ng et al., *Nat. Genet.* **42**, 30 (2010).
10. Materials and methods are available as supporting material on Science Online.
11. D. E. Jensen et al., *Oncogene* **16**, 1097 (1998).
12. S. Misaghi et al., *Mol. Cell. Biol.* **29**, 2181 (2009).
13. H. Nishikawa et al., *Cancer Res.* **69**, 111 (2009).
14. Y. J. Machida, Y. Machida, A. A. Vashisht, J. A. Wohlschlegel, A. Dutta, *J. Biol. Chem.* **284**, 34179 (2009).
15. S. Tyagi, A. L. Chabes, J. Wysocka, W. Herr, *Mol. Cell* **27**, 107 (2007).

16. A. G. de Ayala Alonso et al., *Genetics* **176**, 2099 (2007).
17. J. C. Scheuermann et al., *Nature* **465**, 243 (2010).
18. L. D. Wood et al., *Science* **318**, 1108 (2007).
19. M. D. Onken et al., *Cancer Res.* **66**, 4602 (2006).
20. M. D. Onken, L. A. Worley, J. P. Ehlers, J. W. Harbour, *Cancer Res.* **64**, 7205 (2004).
21. S. Misaghi et al., *J. Biol. Chem.* **280**, 1512 (2005).
22. Y. Wang et al., *Nucleic Acids Res.* **35**, D298 (2007).
23. This work was supported by grants to J.W.H. from the National Cancer Institute (R01 CA125970), Barnes-Jewish Hospital Foundation, Kling Family Foundation, Tumori Foundation, Horncrest Foundation, and a Research to Prevent Blindness David F. Weeks Professorship, and by awards to the Department of Ophthalmology and Visual Sciences at Washington University from a Research to Prevent Blindness, Inc., unrestricted grant, and the NIH Vision Core Grant P30 EY02687c. E.D.O.R. was supported by NIH National Institute of Arthritis and Musculoskeletal and Skin Diseases training grant AR007279-31A1. We thank J. Hoisington-Lopez from the Center for Genome Sciences (Washington University School of Medicine) for running Solexa paired end sequences and M. Lovett for comments on the manuscript. J.W.H. and Washington University may receive income based on a license of related technology by the university to Castle Biosciences, Inc.

#### Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1194472/DC1  
Materials and Methods  
Figs. S1 to S5  
Tables S1 to S3  
References

30 June 2010; accepted 25 October 2010  
Published online 4 November 2010;  
10.1126/science.1194472

## Direct Exchange of Electrons Within Aggregates of an Evolved Syntrophic Coculture of Anaerobic Bacteria

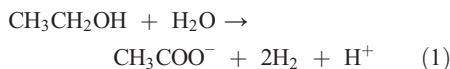
Zarath M. Summers,<sup>1</sup> Heather E. Fogarty,<sup>1</sup> Ching Leang,<sup>1</sup> Ashley E. Franks,<sup>1</sup>  
Nikhil S. Malvankar,<sup>1,2</sup> Derek R. Lovley<sup>1\*</sup>

Microbial consortia that cooperatively exchange electrons play a key role in the anaerobic processing of organic matter. Interspecies hydrogen transfer is a well-documented strategy for electron exchange in dispersed laboratory cultures, but cooperative partners in natural environments often form multispecies aggregates. We found that laboratory evolution of a coculture of *Geobacter metallireducens* and *Geobacter sulfurreducens* metabolizing ethanol favored the formation of aggregates that were electrically conductive. Sequencing aggregate DNA revealed selection for a mutation that enhances the production of a c-type cytochrome involved in extracellular electron transfer and accelerates the formation of aggregates. Aggregate formation was also much faster in mutants that were deficient in interspecies hydrogen transfer, further suggesting direct interspecies electron transfer.

Interspecies exchange of metabolites is crucial to the balanced functioning of many microbial communities (1–5). The canonical example of an essential microbial exchange

is interspecies hydrogen transfer, in which two cell types, neither of which is independently capable of anaerobically oxidizing an organic compound, cooperatively exchange electrons through the production and consumption of hydrogen in order to degrade the substrate (4, 5). The concept of interspecies hydrogen transfer was developed from studies of *Methanobacillus omelianskii*, a coculture of the “S organism” that converted ethanol to acetate and hydrogen gas (Eq. 1), and

the methanogen *Methanobacterium ruminantium*, which consumed hydrogen with the reduction of carbon dioxide to methane (Eq. 2) (6):

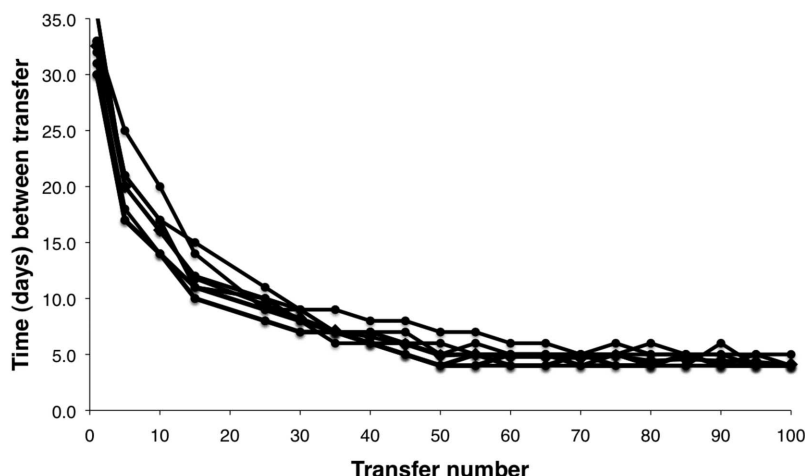


The S organism is no longer available in culture, but *Pelobacter carbinolicus* functions similarly (7). *P. carbinolicus* evolved from Fe(III)-reducing members of the Geobacteraceae family to grow as an ethanol-oxidizing syntroph (8). When Fe(III)-reducing microorganisms deplete Fe(III) in anaerobic soils and sediments, it is advantageous for them to form syntrophic associations with microorganisms that can accept the electrons that were formerly transferred to Fe(III).

To investigate how Fe(III) reducers in the Geobacteraceae family switch to syntrophic growth, we initiated nine replicate cocultures (9) with *Geobacter metallireducens*, an ethanol-oxidizing Fe(III) reducer closely related to *P. carbinolicus* (10). *Geobacter sulfurreducens* (11), which cannot metabolize ethanol, was added as a hydrogen-consuming partner with fumarate as the electron acceptor, which *G. metallireducens* cannot use. In such a coculture, *G. sulfurreducens* could presumably make ethanol metabolism by *G.*

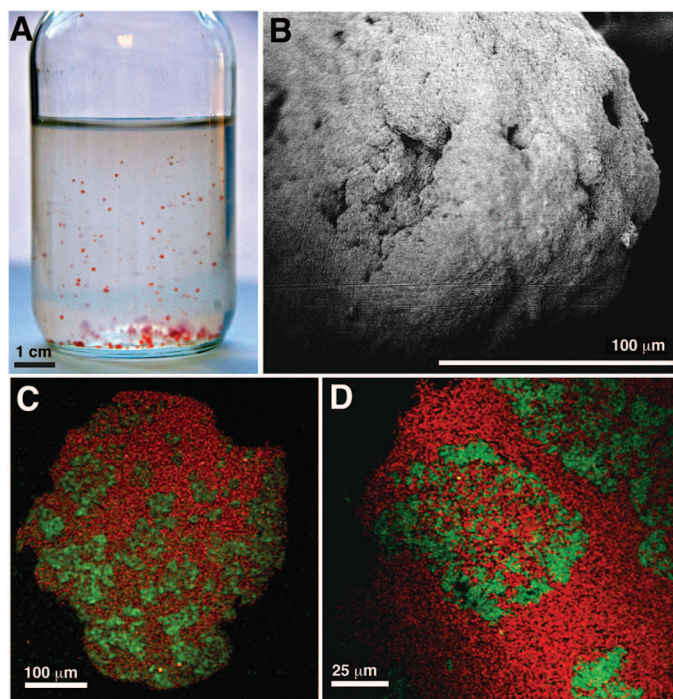
<sup>1</sup>Department of Microbiology, University of Massachusetts, Amherst, MA 01003, USA. <sup>2</sup>Department of Physics, University of Massachusetts, Amherst, MA 01003, USA.

\*To whom correspondence should be addressed. E-mail: dlovley@microbio.umass.edu

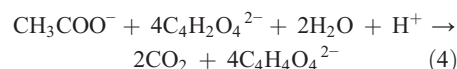
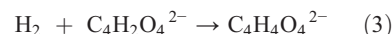


**Fig. 1.** Time required for sequential transfers of each of the individual cocultures of *G. metallireducens* and *G. sulfurreducens* to metabolize at least 70% of the ethanol provided.

**Fig. 2.** Aggregates in evolved coculture of *G. metallireducens* and *G. sulfurreducens*. (A) Aggregates in culture bottle. (B) Scanning electron micrograph of a typical aggregate. (C) FISH of a semi-thin section of an aggregate treated with green-fluorescing *G. metallireducens* probes and red-fluorescing *G. sulfurreducens* probe. (D) FISH analysis at higher magnification.



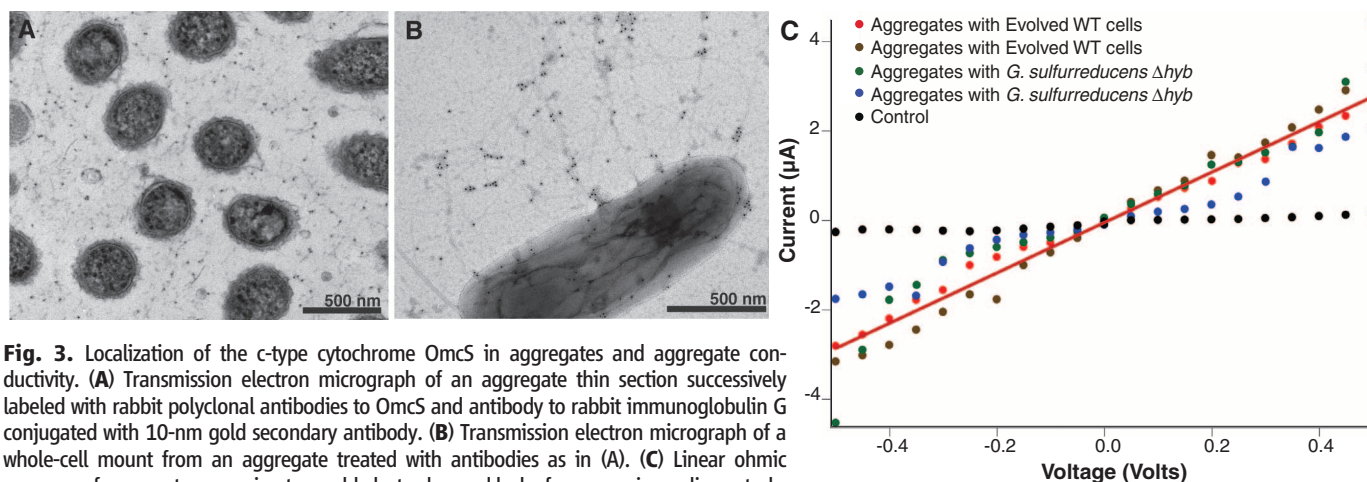
*metallireducens* possible by consuming the hydrogen produced by *G. metallireducens* with the reduction of fumarate to succinate (Eq. 3); *G. sulfurreducens* would also consume the acetate that *G. metallireducens* produced from ethanol (Eq. 4):



Initially the coculture grew very slowly and required about 30 days to metabolize ~70% of the ethanol provided. During this phase, measured hydrogen concentrations in the culture were ~10 parts per million (ppm). With continued transfer (1% inoculum) into fresh medium after the cocultures had metabolized ~70% of the ethanol, the coculture adapted to consume at least 70% of the ethanol within 4 days (Fig. 1). This increase in metabolic rate was accompanied by the formation of large spherical aggregates (diameter ~1 to 2 mm) that began to appear as small flocks by transfer 12 and had formed large, tight spherical associations by transfer 30 (Fig. 2A). Hydrogen concentrations were ~6 ppm at this stage. The aggregates were morphologically similar to those that typically form during anaerobic treatment of wastewater (12) with channels to promote exchange of materials with the bulk environment (Fig. 2B).

Quantitative polymerase chain reaction targeting 16S rRNA gene sequences indicated that *G. metallireducens* accounted for ~15% of the cells in the aggregates (fig. S1). Examination of thin sections of the aggregates by fluorescence in situ hybridization (FISH) with species-specific oligonucleotide probes revealed that the two species formed distinct clusters within the aggregates (Fig. 2, C and D).

To evaluate the role of interspecies hydrogen transfer within the aggregates, we initiated cocultures with a strain of *G. sulfurreducens* in which the gene *hyb* was deleted. This gene encodes a hydrogenase subunit, and previous studies have shown that the strain with *hyb* deleted is unable



**Fig. 3.** Localization of the c-type cytochrome OmcS in aggregates and aggregate conductivity. (A) Transmission electron micrograph of an aggregate thin section successively labeled with rabbit polyclonal antibodies to OmcS and antibody to rabbit immunoglobulin G conjugated with 10-nm gold secondary antibody. (B) Transmission electron micrograph of a whole-cell mount from an aggregate treated with antibodies as in (A). (C) Linear ohmic response of aggregates spanning two gold electrodes, and lack of response in media controls.



to consume hydrogen (13). The coculture metabolized ethanol (fig. S2). Furthermore, it formed large aggregates within 21 days, rather than the 7 months required for the cocultures containing wild-type *G. sulfurreducens*. Interspecies formate transfer is a potential alternative to interspecies hydrogen transfer for electron exchange between some organisms (5), but this was not a possibility in the *Geobacter* coculture because *G. sulfurreducens* is unable to use formate as an electron donor (11), and aggregates were incapable of formate-dependent fumarate reduction (fig. S3). These results suggest that an alternative mechanism of electron transfer between the two *Geobacter* species, enhanced by close cell association, confers a growth advantage when interspecies hydrogen transfer is no longer possible.

Sequencing of genomic DNA extracted from one of the aggregate cultures evolved from wild-type cells after the 15th transfer (~100 cell generations) revealed a single mutation in *G. sulfurreducens* and none in *G. metallireducens*. The mutation in *G. sulfurreducens*, which was also present in the other eight replicate cocultures but was not detected in the common inoculum, was a deletion of a single base pair in the gene encoding PilR, which functions as an RpoN-dependent enhancer-binding protein and regulates the expression of a variety of genes in *G. sulfurreducens* (14). This deletion resulted in a frame shift changing the amino acids starting at position 337 and introduced a stop codon at position 342, resulting in a truncated PilR protein that lacked the helix-turn-helix domain required for DNA binding (fig. S4). Although this mutation was not detected in the common inoculum used to initiate the cocultures, it is conceivable that it was a rare variant in that culture. Furthermore, genome resequencing may fail to detect transposition or other genome rearrangements. Therefore, to determine whether a mutation in *pilR* was sufficient to promote rapid syntrophic metabolism, we initiated the cocultures with a strain of *G. sulfurreducens* in which *pilR* was deleted. Within 21 days, all three replicate cocultures formed aggregates in the initial culture tubes, which suggests that inactivation of PilR was sufficient to promote aggregate formation.

One of the primary impacts of deleting *pilR* in *G. sulfurreducens* is enhanced expression of the gene encoding OmcS (14), a multiheme c-type cytochrome that promotes electron transfer to insoluble Fe(III) oxides (15) and electrodes (16). OmcS is primarily associated with pili (17). The multiple hemes in OmcS are thought to facilitate the transfer of electrons exported from the cell along the pili, which are electrically conductive (18, 19), onto electron acceptors, such as Fe(III) oxide (17). Immunogold labeling of OmcS revealed that OmcS was dispersed throughout

the intercellular matrix (Fig. 3A) and was associated with pili (Fig. 3B). Heme staining and Western blot analysis indicated that OmcS was the most abundant c-type cytochrome in the aggregates and that OmcS was much more abundant in the evolved aggregates than in monocultures of *G. sulfurreducens* (fig. S5). Deletion of the genes encoding OmcS or PilA, the structural pilin protein, prevented any visible growth even after long-term incubation (>9 months).

These results show that OmcS is essential for effective syntrophic electron exchange and that selective pressure for syntrophic growth selected for a mutation that enhanced OmcS production. The lack of detailed study of *G. metallireducens* and the lack of homology between outer-surface cytochromes in the *Geobacter* species (20) has precluded definitive verification of the mechanisms for extracellular electron transfer in *G. metallireducens*, but it is expected that *G. metallireducens* also uses a network of conductive pili and cytochromes, similar to the closely related *G. sulfurreducens*. Thus, a likely model for electron exchange between *G. sulfurreducens* and *G. metallireducens* is that OmcS of *G. sulfurreducens* can accept electrons from outer-surface c-type cytochromes of *G. metallireducens* that are either localized on the cell or along pili. This direct exchange of electrons would alleviate the need for interspecies hydrogen transfer and would explain the effective syntrophy between *G. metallireducens* and *G. sulfurreducens*, even when *G. sulfurreducens* was unable to consume hydrogen. Indeed, the aggregates exhibited ohmic conductance when placed between two gold electrodes (Fig. 3C and fig. S6).

Our results suggest that selective pressure for effective electron exchange in this coculture favored direct electron transfer between consortium members, rather than interspecies hydrogen transfer. This is consistent with previous speculation on the possibility of direct electron transfer between cells (18, 21), but in the absence of experimental evidence, this possibility has been met with skepticism (4, 5), especially considering the preponderance of evidence from studies with defined cocultures in which interspecies transfer of hydrogen or formate predominates. Previous coculture studies, however, typically examined dispersed (i.e., not aggregated) syntrophic partners (22). The lack of cell aggregation dictates that interspecies hydrogen or formate transfer predominates over direct cell-to-cell electron transfer under those conditions. In contrast, aggregation of cells involved in syntrophic anaerobic metabolism appears to be the rule in most environments (1, 3, 23) and is key to the successful anaerobic treatment of wastewater (12). Modeling of electron transfer (22, 24–26) in such aggregates, as well as experimental evidence

(26, 27), suggests that interspecies hydrogen transfer is not the primary mechanism for electron exchange. Rapid direct electron exchange is consistent with the findings in those studies, and in fact, extracellular c-type cytochromes may play an important role in aggregates catalyzing syntrophic methane oxidation (28).

## References and Notes

1. C. J. Marx, *Science* **324**, 1150 (2009).
2. K. L. Hillesland, D. A. Stahl, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 2124 (2010).
3. K. Knittel, A. Boetius, *Annu. Rev. Microbiol.* **63**, 311 (2009).
4. M. J. McInerney, J. R. Sieber, R. P. Gunsalus, *Curr. Opin. Biotechnol.* **20**, 623 (2009).
5. A. J. Stams, C. M. Plugge, *Nat. Rev. Microbiol.* **7**, 568 (2009).
6. M. P. Bryant, E. A. Wolin, M. J. Wolin, R. S. Wolfe, *Arch. Microbiol.* **59**, 20 (1967).
7. B. Schink, in *The Prokaryotes*, M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, E. Stackebrandt, Eds. (Springer, New York, 2006), vol. 7, pp. 5–11.
8. J. E. Butler, N. D. Young, D. R. Lovley, *BMC Genomics* **10**, 103 (2009).
9. See supporting material on Science Online.
10. D. R. Lovley et al., *Arch. Microbiol.* **159**, 336 (1993).
11. F. Caccavo Jr., et al., *Appl. Environ. Microbiol.* **60**, 3752 (1994).
12. J. E. Schmidt, B. K. Ahning, *Biotechnol. Bioeng.* **49**, 229 (1996).
13. M. V. Coppi, R. A. O'Neil, D. R. Lovley, *J. Bacteriol.* **186**, 3022 (2004).
14. K. Juárez et al., *J. Mol. Microbiol. Biotechnol.* **16**, 146 (2009).
15. T. Mehta, M. V. Coppi, S. E. Childers, D. R. Lovley, *Appl. Environ. Microbiol.* **71**, 8634 (2005).
16. D. E. Holmes et al., *Environ. Microbiol.* **8**, 1805 (2006).
17. C. Leang, X. Qian, T. Mester, D. R. Lovley, *Appl. Environ. Microbiol.* **76**, 4080 (2010).
18. G. Reguera et al., *Nature* **435**, 1098 (2005).
19. G. Reguera et al., *Appl. Environ. Microbiol.* **72**, 7345 (2006).
20. J. E. Butler, N. D. Young, D. R. Lovley, *BMC Genomics* **11**, 40 (2010).
21. Y. A. Gorby et al., *Proc. Natl. Acad. Sci. U.S.A.* **103**, 11358 (2006).
22. S. S. Oztürk, B. Ø. Palsson, J. H. Thiele, *Biotechnol. Bioeng.* **33**, 745 (1989).
23. B. Kartal, J. G. Kuenen, M. C. van Loosdrecht, *Science* **328**, 702 (2010).
24. M. J. Alperin, T. M. Hoehler, *Am. J. Sci.* **309**, 869 (2009).
25. B. Orcutt, C. Meile, *Biogeosciences* **5**, 1587 (2008).
26. K. B. Sørensen, K. Finster, N. B. Ramsing, *Microb. Ecol.* **42**, 1 (2001).
27. K. Nauhaus, A. Boetius, M. Krüger, F. Widdel, *Environ. Microbiol.* **4**, 296 (2002).
28. A. Meyerdierts et al., *Environ. Microbiol.* **12**, 422 (2010).
29. We thank P. Brown, C. Barrett, K. Zengler, and Y. Qiu for sequence analysis; L. Raboin for SEM assistance; and K. Rubin for photography assistance. Supported by the Office of Science (Office of Biological and Environmental Research), U.S. Department of Energy, Cooperative Agreement DE-FC02-02ER63446 and Award DE-FC-0004485.

## Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6009/1413/DC1  
Materials and Methods  
Figs. S1 to S5  
References

16 August 2010; accepted 22 October 2010  
10.1126/science.1196526



### MICROPLATE READER

The Synergy H1 Hybrid is offered as a highly flexible, high value monochromator-based multi-mode microplate reader for ultraviolet-visible absorbance, top and bottom fluorescence, and luminescence assays. The modular architecture can be easily upgraded to a “hybrid” reader with the addition of a high performance filter module, offering true versatility and unlimited assay use. This independent filter module with dichroic mirrors supports fluorescence polarization (FP), time-resolved fluorescence (TRF), TR-FRET, and BRET assays with ultrahigh sensitivity. Synergy H1 includes the Gen5 Data Analysis Software for advanced reader control, data analysis, graphing, exporting, and reporting. Additionally, Synergy H1 is compatible with the Take3 Multi-Volume Plate that measures up to sixteen 2- $\mu$ l samples, two BioCells, or a standard cuvette for fast and simple multivolume, multisample analysis.

BioTek Instruments, Inc.

For info: 888-451-5171 | [www.biotek.com](http://www.biotek.com)

### CELL HEALTH ANALYSIS

FlowCelect kits are designed for cell health analysis using flow cytometry and allow researchers to quickly and easily evaluate mitochondrial health in drug compound screening, apoptosis-related research, and understanding the mechanism of diseases. The six new kits include the MitoDamage kit, which provides simultaneous information on mitochondrial perturbations, apoptosis, and cell death. The MitoStress kit allows study of the interrelationship between mitochondrial oxidative stress and apoptosis. For researchers who want to further understand mechanistic pathways, Cytochrome C kits allow for evaluation of mitochondrial Cytochrome C levels in apoptotic cells and commitment to the intrinsic pathway of death, thereby simplifying an assay that has traditionally been done with Western blots. The kits may be used on any flow cytometer equipped with blue and red lasers.

EMD Millipore

For info: 800-645-54767 | [www.millipore.com/mitohealth](http://www.millipore.com/mitohealth)

### LOW EVAPORATION PLATES

Combining advanced optical properties with a low evaporation rate, Nunc Edge Plates enable researchers to obtain quality data from automated fluorescence imaging and quantitative analysis protocols. These plates incorporate large perimeter evaporative buffer zones that eliminate well-to-well variability, while dramatically reducing the overall plate evaporation rate to less than two percent after seven days of incubation. As a result, more viable and healthy cell yields are obtained. These zones enable the use of all 96 wells, greatly reducing the edge effect commonly experienced in cell culture, while maintaining data consistency throughout the plate. Nunc Edge Plates significantly reduce volume loss, effectively maintaining sample concentrations over long periods of incubation. The prevention of cell death and toxicity in the plates' outer wells allows results to remain more true to the population phenotype for more efficient, high throughput analysis. Nunc Edge plates offer outstanding flatness and superior image quality, ideal for high content analysis applications and cell-based assays where multiple targets are simultaneously imaged.

Thermo Fisher Scientific

For info: 800-625-4327 | [www.thermoscientific.com/edgeplate](http://www.thermoscientific.com/edgeplate)

### MICROPATTERNED SUBSTRATES

Patterns of extracellular matrix proteins can control important cellular functions including mitosis, apoptosis, cell growth, stem cell differentiation, tissue growth and structure, and multicellular dynamics and interactions. The new BioWrite line and grid protein micropatterns on glass coverslips are designed for cell-based applications. The fibronectin patterns have feature sizes ranging from 15  $\mu$ m to 100  $\mu$ m, which restrict the sites of cellular adhesion and spreading. The protein patterns can define the location, size, and morphology of cultured cells; control cellular functions; direct migration; and minimize variability in cell-based assays. The Substrate Design Center enables researchers to rapidly develop custom protein micropatterns with features as small as 5  $\mu$ m for specific cell-based applications. These products and services provide improved sensitivity, lower variability, and simpler automated image analysis for next generation cell-based assays.

Intelligent Substrates

For info: 410-982-6609 | [www.intelligentsubstrates.com](http://www.intelligentsubstrates.com)

### CELL CULTURE SEPARATION

FlexiPERM reusable silicone inserts can be used to subdivide slides and any cell culture dish into smaller cultivation units. Different cell lines can be grown in one vessel or equivalent cell lines can be grown in parallel with the assurance of comparable growth conditions. FlexiPERM inserts are highly adhesive to any flat surface for up to 14 days without additional—potentially contaminating—adhesives. After use, these inserts are easily removed without tools and may be sterilized and reused at least 10 times. Five versions of flexiPERM inserts are available. The flexiPERM slide and the flexiPERM micro 12 are ideally sized for microscope slides and feature eight and 12 cultivation wells, respectively. The flexiPERM disc provides four cultivation units and is appropriate for use with 60 mm dishes. FlexiPERM conA and conB inserts are single cone-shaped chambers suitable for micromanipulation and micro-injection applications.

Sarstedt

For info: 800-257-5101 | [www.sarstedt.com](http://www.sarstedt.com)

Electronically submit your new product description or product literature information! Go to [www.sciencemag.org/products/newproducts.dtl](http://www.sciencemag.org/products/newproducts.dtl) for more information.

Newly offered instrumentation, apparatus, and laboratory materials of interest to researchers in all disciplines in academic, industrial, and governmental organizations are featured in this space. Emphasis is given to purpose, chief characteristics, and availability of products and materials. Endorsement by *Science* or AAAS of any products or materials mentioned is not implied. Additional information may be obtained from the manufacturer or supplier.



## Multiply The Power of Science



### Science Careers Classified Advertising

For full advertising details, go to [ScienceCareers.org](http://ScienceCareers.org) and click For Employers, or call one of our representatives.

#### Tracy Holmes

Worldwide Associate Director  
Science Careers  
Phone: +44 (0) 1223 326525

#### UNITED STATES & CANADA

E-mail: [advertise@sciencecareers.org](mailto:advertise@sciencecareers.org)  
Fax: 202-289-6742

#### Tina Burks

Midwest/West Coast/  
South Central/Canada  
Phone: 202-326-6577

#### Elizabeth Early

East Coast & Industry  
Phone: 202-326-6578

#### Marci Gallun

Sales Administrator  
Phone: 202-326-6582

#### Online Job Posting Questions

Phone: 202-326-6577

#### EUROPE & REST OF WORLD

E-mail: [ads@science-int.co.uk](mailto:ads@science-int.co.uk)  
Fax: +44 (0) 1223 326532

#### Alex Palmer

Phone: +44 (0) 1223 326527

#### Susanne Kharraz Tavakol

Phone: +44 (0) 1223 326529

#### Dan Pennington

Phone: +44 (0) 1223 326517

#### Lisa Patterson

Phone: +44 (0) 1223 326528

#### JAPAN

#### ASCA Corporation

Jie Chin  
Phone: +81-3-6802-4616  
Fax: +81-3-6802-4615  
E-mail: [careerads@sciencemag.jp](mailto:careerads@sciencemag.jp)

#### To subscribe to Science:

In United States call 866-434-2227  
In the rest of the world call +1 202-326-6417

All ads submitted for publication must comply with applicable U.S. and non-U.S. laws. *Science* reserves the right to refuse any advertisement at its sole discretion for any reason, including without limitation for offensive language or inappropriate content, and all advertising is subject to publisher approval. *Science* encourages our readers to alert us to any ads that they feel may be discriminatory or offensive.

**Science Careers**

From the journal *Science* 

## POSITIONS OPEN



### TWO ASSISTANT PROFESSORSHIPS Systems Biology and Cell & Molecular Biology University of California, Merced

The School of Natural Sciences at the University of California, Merced seeks applicants for two tenure-track assistant professor positions in the areas of Systems Biology and Cell & Molecular Biology (CMB). Candidates are expected to have an excellent publication record and to teach biology effectively at both undergraduate and graduate levels. Systems Biology is used here to mean a research approach that uses comprehensive datasets and multiple types of analysis to relate overall biological function to the underlying biochemical or biophysical processes, with an ultimate goal of a predictive understanding. For the CMB search priority will be given to candidates studying the molecular and cellular mechanisms of biological processes in model organisms. For both positions, applications of special relevance to research emphases at UC Merced include the mechanisms of cell fate decisions, complex diseases such as diabetes or inflammatory disorders, and microbial systems relevant to human disease. For more information, see [website: http://jobs.ucmerced.edu/n/academic/listings.jsf?seriesId=10](http://webiste: http://jobs.ucmerced.edu/n/academic/listings.jsf?seriesId=10).

Interested applicants should submit online: curriculum vitae, statements of research and teaching interests, and the names and addresses of five references. Applications will be considered starting on November 25, 2010. *UC Merced is an AA/EOP employer.*

### BIO-COMPUTER SCIENTIST

Wilkes University invites applications for a tenure-track **ASSISTANT PROFESSOR** appointment in the College of Science and Engineering starting fall 2011. This position is in part sponsored by the Howard Hughes Medical Institute to enhance quantitative skills of undergraduates in the life sciences. We seek a candidate who can teach and conduct research at the interface between biology and computer science in an undergraduate setting. The applicant must have a Ph.D. in a relevant field; postdoctoral experience is preferred. Potential areas of specialty include but are not limited to mathematical modeling and simulation, bioinformatics, computational biology, epidemiology, or geospatial statistics.

The successful candidate will develop a strong research program involving undergraduates and will participate in efforts to provide a strong interface between Biology and CS. Teaching responsibilities will include participation in introductory and upper-level undergraduate courses appropriate to the candidate's areas of expertise. A competitive startup package is available.

Wilkes University is an independent institution of higher education with approximately 2,200 undergraduates located in Wilkes-Barre, Pennsylvania, a mid-sized city within two and one-half hours driving distance of New York City and Philadelphia. The new hire will join a productive and collaborative cluster of computational scientists from mathematics, computer science, biology, and chemistry.

Please send application letter, curriculum vitae including contact information for three references, research and teaching statements, and recent publications in PDF format. For full consideration, submit all materials by January 7, 2011, to e-mail: [william.terzaghi@wilkes.edu](mailto:william.terzaghi@wilkes.edu). *Wilkes University is an Equal Opportunity employer committed to a diverse faculty, staff and student body. Applicants from diverse backgrounds are strongly encouraged to apply.*

For further information about this position, please contact the Chair of the search committee, **Dr. William Terzaghi** at e-mail: [william.terzaghi@wilkes.edu](mailto:william.terzaghi@wilkes.edu).

## POSITIONS OPEN



### POSTDOCTORAL POSITIONS Texas A&M Institute for Regenerative Medicine at Temple, TX

The Texas A&M Institute for Regenerative Medicine is seeking **POSTDOCTORAL FELLOWS** for research on adult stem/progenitor cells. The Institute is dedicated to research both on the basic biology of stem/progenitor cells and their potentials for therapies of human diseases. It occupies newly created laboratories and a series of core laboratories in an expanding academic medical center. Postdoctoral appointments will be for one year with the opportunity to renew for a second or third year subject to performance. Please send curriculum vitae, brief statement of research interests, and three recommendation letters to the attention of **Darwin J. Prockop, M.D., Ph.D., Director, Texas A&M Institute for Regenerative Medicine** at e-mail: [regenerate@medicine.tamhsc.edu](mailto:regenerate@medicine.tamhsc.edu). *The Texas A&M Health Science Center is an Affirmative Action/Equal Opportunity Employer.*

### ASSISTANT OR ASSOCIATE PROFESSOR

The Division of Musculoskeletal Sciences, Department of Orthopaedics and Rehabilitation at The Pennsylvania State University, College of Medicine announces a search for an Assistant or Associate Professor (tenured or tenure-track) in the area of musculoskeletal sciences or engineering. This position includes a highly competitive salary and significant startup funds. The successful candidate will be an individual who can apply modern biomedical science and engineering concepts to the study of musculoskeletal tissues. This is a unique opportunity to join a well-established, highly interactive research group consisting of engineers, material, clinical, and basic scientists focusing on musculoskeletal research. A joint academic appointment will include a primary appointment in the College of Medicine and a secondary appointment in an appropriate department at Penn State. The Penn State College of Medicine is located in Hershey, Pennsylvania and offers a highly desirable lifestyle and an affordable cost of living in close proximity to many metropolitan areas including Baltimore, Washington, D.C., Philadelphia, and New York City. Applications will be accepted until the position is filled.

Send curriculum vitae to: **Ananya Das, Faculty Search Committee, Division of Musculoskeletal Sciences, Department of Orthopaedics and Rehabilitation, The Pennsylvania State University, College of Medicine, 500 University Drive, H089, Hershey, PA 17033.**

*Penn State is committed to Affirmative Action, Equal Opportunity, and the diversity of its workforce.*

### TENURE-TRACK FACULTY POSITIONS in Cardiovascular Physiology, Metabolism, Stress

The Department of Integrative Biology and Physiology at the University of Minnesota Medical School seeks outstanding faculty candidates in integrative systems biology of the cardiovascular system, metabolism, and stress open to all ranks (**ASSISTANT, ASSOCIATE, FULL PROFESSOR**). Successful candidates will have an innovative research program that embraces biological complexity from molecular building blocks to the living organism with a focus on cardiovascular biology, metabolic syndrome, diabetes, obesity, and stress. A commitment to excellence in teaching is essential. Minimum requirements are a Ph.D., M.D., or M.D.-Ph.D. with two or more years of postdoctoral training. Applicants should apply online ([website: http://employment.umn.edu](http://employment.umn.edu); requisition number 169479) and submit curriculum vitae, cover letter, and references. *The University of Minnesota is an Equal Opportunity Educator and Employer.*



## Postdoctoral Fellowships in Genomic Biology at the University of Illinois at Urbana-Champaign

The Institute for Genomic Biology at the University of Illinois at Urbana-Champaign offers a number of postdoctoral fellowships for talented young scholars. IGB Fellows spend two to three years doing collaborative and independent research in one of several research themes at the Institute. Visit [www.igb.illinois.edu/people/igb-fellows](http://www.igb.illinois.edu/people/igb-fellows) for more information about the Institute, the research themes, and application procedures. The closing date for all positions is February 1, 2011. Fellows will be announced on or before March 15, 2011.

### Biocomplexity

We seek a quantitative scientist with interests in evolution, systems biology, and ecosystem dynamics and expertise in statistical physics as applied to biology. The successful candidate will join a multidisciplinary group exploring collective effects in biology. Projects include the evolution of translation, the role of horizontal gene transfer in communities of microbes and phages, and the systems biology of cells and ecosystems. (Nigel Goldenfeld, Theme Leader)

### Regenerative Biology and Tissue Engineering

The Fellow will be involved in one or more of our multidisciplinary projects related to regenerative biology and harnessing the potential of adult/embryonic stem cells for tissue engineering applications. Of particular interest is leveraging theme expertise in biomaterials fabrication, drug delivery systems, microfluidics-based *in vitro* experimental platforms, and *in vivo* evolutionary biology and regeneration medicine studies. The ideal candidate will have experience in one or more areas of (stem) cell biology, induced pluripotent cell technology, biomaterials, microfluidics, and/or tissue engineering. (Paul Kenis, Theme Leader)

### Genomic Ecology of Global Change

The Fellow will be involved in a cross-disciplinary project investigating how changes in networks of genes affect ecosystem metabolism when challenged by elements of global change, including elevated atmospheric carbon dioxide and ozone, increased drought, and altered interactions with insect herbivores and plant pathogens. The ideal candidate will have a strong background in plant biology and a record of expertise in molecular biology, genomic ecology, physiology or modeling of gene networks, or ecosystem function. The ability to work creatively and productively in a highly interdisciplinary and collaborative environment is essential. (Don Ort, Theme Leader)

### Molecular Bioengineering of Biomass Conversion

We seek an individual with experience in metabolic engineering of industrially significant microbes (e.g. yeast, fiber-degrading bacteria, clostridia) for production of biofuels from biomass feedstocks. The ideal candidate will have expertise in modern molecular biology techniques, whole genome shuffling, DNA microarrays, proteomic and metabolic tools, transposon mutagenesis, and high-throughput screening methods to maximize the production of bioproducts and biofuels. (Hans Blaschek, Theme Leader)

### Energy Biosciences Institute

The Energy Biosciences Institute (EBI) is an externally funded theme within the IGB. It is the largest academia collaboration to date, currently receiving \$500 million over 10 years and focusing on the development of second-generation biofuels intended to significantly slow the rate of global climate change. Its research ranges from systems biology of fermentative organisms to quantification of ecosystem services provided by new sustainable biofuel crops. The full range of research can be seen at [www.energybiosciencesinstitute.org](http://www.energybiosciencesinstitute.org). We seek an outstanding candidate across these areas interested in applying genomic biology to understanding and developing opportunities for improving sustainable biofuel production. Research can be at any point in the supply chain from improving feedstocks and their environmental sustainability to producing fuel. The appointee will work in an interdisciplinary laboratory of more than 100 exceptional colleagues focused on this challenge. The appointment also would involve collaboration with our partners: UC Berkeley and BP. (Steve Long, Theme Leader)

### Cellular Decision Making in Cancer

We seek an individual with interest in quantitative biology. Our theme faculty members have expertise in single molecule biophysics, genomics, and chemical biology. Building on the current strengths in cell death, antiviral signaling, stem cell differentiation, live cell probing of decision making and genome instability modeling, we aim to develop a multiple-scale narrative on how single molecule events in the cell are integrated into the protein networks to determine the cell fate. Cancer is a major focus area of research. (Taekjip Ha, Theme Leader)

### Genomics of Neural and Behavioral Plasticity

We seek a biologist with strong bioinformatics skills and training in one or more of the following areas: evolutionary biology, neuroscience, animal behavior, molecular biology, genomics, or systems biology. Applicants with expertise in both biology and bioinformatics will be strongly preferred. The successful candidate will join a multidisciplinary team that is using genomics to identify both conserved and novel mechanisms of neural and behavioral plasticity in diverse animal systems. Fellows are expected to conduct research that contributes to the development of the theme's goals by integrating components from theme members' individual research programs. (Gene Robinson, Theme Leader)

### Host-Microbe Systems

The Fellow will be responsible for developing DNA isolation, microbial isolation, 16S rRNA gene sequencing, and other metagenomic analysis techniques for surveying microbial content of the human and nonhuman primate vaginal and intestinal microbiomes. Additional responsibilities will include culture isolation, genome sequencing, and other molecular biology techniques to examine microbial, metabolic, and immunologic contents, as well as performing phylogenetic comparisons and analyses using bioinformatics and other computational and analytical methods. The ideal candidate will have a strong background in microbiology, biochemistry, or a related field with experience and expertise in molecular microbial ecology and bioinformatics and/or biostatistics. (Brenda Wilson, Theme Leader)

### Mining Microbial Genomes for Novel Antibiotics

The Fellow will be involved in a multidisciplinary project aimed at the discovery, design, and development of phosphonic acid antibiotics. The ideal candidate will have a proven record of expertise in the general area of microbially produced natural products, with specific expertise in one of several disciplines. We are interested in candidates with previous experience in bacterial metabolism, bacterial genetics, molecular biology, biochemistry, enzyme evolution, metabolic engineering, organic synthesis, mass spectroscopy, bioinformatics, and metagenomics. (Bill Metcalf, Theme Leader)

### Business, Economics, and Law of Genomic Biology

We seek an individual with training in economics, business, law, or strategy and with an interest in technology entrepreneurship, technology industries, and biotechnology. The Fellow will join a multidisciplinary group that includes business, law, and technology experts, agricultural economics faculty, and personnel from the campus Office of Technology Management. Our theme is exploring issues in university-industry technology transfer, industry evolution, intellectual property protection, the competitive and cooperative dynamics for both entrepreneurial start-ups and existing corporations, the impact that globalization of biotechnology has on the evolution of industry, and the position of U.S. firms in the global marketplace. (Jay Kesan, Theme Leader)

**The University of Illinois is an Affirmative Action/Equal Opportunity Employer**

[www.igb.illinois.edu](http://www.igb.illinois.edu)





## HARVARD UNIVERSITY Assistant/Associate Professors Department of Genetics and Complex Diseases

The Department of Genetics and Complex Diseases (GCD) at the Harvard School of Public Health (HSPH) invites applications for tenure-track positions at the level of assistant professor. Exceptional associate professor candidates will also be considered. Successful applicants will hold a PhD and/or MD degree and will have a record of outstanding productivity. Individuals are sought particularly in the following areas to complement the existing research and training goals of the department: signal transduction related to energy and nutrient sensing pathways, regulation of metabolic homeostasis, inflammatory and stress response pathways related to chronic metabolic diseases and aging, cancer metabolism, and epigenetic regulation of metabolism. Individuals using systems and/or computational approaches applied at a mechanistic level to problems of metabolic homeostasis, gene-environment interactions and/or adaptive responses are also encouraged to apply. The candidate should possess the ability to work collaboratively with other scientists and the scholarly qualities required to mentor doctoral students in the graduate program in the Division of Biological Sciences. Generous startup packages and state-of-the-art research facilities are available.

Please send a letter of application, including a statement of current and future research interests, curriculum vitae, sample publications, and the names of four references to the following address. Applicants should ask their four references to write independently to this address.

**Chair, GCD Search, c/o Holly Johnsen**  
**Department of Genetics & Complex Diseases**  
**Harvard School of Public Health**  
**655 Huntington Avenue, Building II, 101**  
**Boston, MA 02115**  
**gcddept@hsph.harvard.edu**

*The Harvard School of Public Health is committed to increasing the representation of women and minorities in its faculty, and encourages applications from such candidates.*



## THE CHINESE UNIVERSITY OF HONG KONG

Applications are invited for:-  
**School of Biomedical Sciences**

**Associate Professors / Assistant Professors (Non-clinical)**

(Ref. 1011/037(665)/2) (Closing date: December 28, 2010)

The School of Biomedical Sciences was established under the Faculty of Medicine in June 2009 through re-organization of its preclinical departments. It is the Faculty's vision to become a world-recognized leader in selected fields of biomedical sciences while maintaining excellence in postgraduate and undergraduate educational programmes. Currently, the School has research programmes focusing on neuro-degeneration, cancer biology and inflammation, developmental biology, reproduction and endocrinology, vascular and metabolic biology, and stem cell and regeneration.

The School invites applications for Associate Professorships / Assistant Professorships. Individuals who are strongly motivated by the biological importance of their research and who value the opportunity to work in close collaboration with others are welcome to apply. Those with expertise in stem cell biology, developmental genomics, and organogenesis are particularly welcome. The School will provide state-of-the-art research facilities, appropriate laboratory and office space, and a start-up package.

Applicants should have (i) a PhD, MD, or DVM degree or equivalent in biological sciences or related disciplines; (ii) experience in using cutting-edge approaches including but not limited to live cell imaging, genetics, genomics, proteomics, bioinformatics, or transgenic animal models in research; and (iii) a good track record of research and publication in international peer-reviewed scientific journals in one or more related areas. The appointees will (a) teach undergraduate, postgraduate, and general education courses; and (b) lead a vigorous independent research programme. Appointments will normally be made on contract basis for up to three years initially commencing May 2011, which, subject to mutual agreement, may lead to longer-term appointment or substantiation later.

### Salary and Fringe Benefits

Salary will be highly competitive, commensurate with qualifications and experience. The University offers a comprehensive fringe benefit package, including medical care, plus a contract-end gratuity for appointments of two years or longer, and housing benefits for eligible appointees. Further information about the University and the general terms of service for appointments is available at <http://www.cuhk.edu.hk/personnel>. The terms mentioned herein are for reference only and are subject to revision by the University.

### Application Procedure

Please send full resume, copies of academic credentials, a publication list and/or abstracts of selected published papers together with names, addresses and fax numbers/e-mail addresses of three referees to whom the applicants' consent has been given for their providing references (unless otherwise specified), to the Personnel Office, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong (Fax: (852) 2696 1462) by the closing date. The Personal Information Collection Statement will be provided upon request. Please quote the reference number and mark 'Application - Confidential' on cover.



## FACULTY POSITIONS in INFECTIOUS DISEASES Tulane National Primate Research Center TULANE UNIVERSITY

The Tulane National Primate Research Center (TNPRC) wishes to expand its infectious disease research programs involving molecular virology, bacteriology, and parasitology by reaching into areas covered by the NIH/NIAID National Biodefense Program, as well as vaccinology and vector-borne and emerging diseases. Agent-specific interests for the expansion include but are not limited to HIV/AIDS; tick-borne bacterial diseases, e.g. Lyme disease; bacterial diseases that affect the respiratory and nervous systems, e.g. tuberculosis and pneumococcal pneumonia; brucellosis; and malaria.

Four positions are available at the rank of Assistant, Associate, or Full Professor. Designations may be either in the Tenure-Track or within the Research Professor Series, depending on qualifications. Academic appointments will be in appropriate Departments of either the Tulane University School of Medicine or the Department of Tropical Medicine of the Tulane School of Public Health and Tropical Medicine. The research portfolio and infrastructure of the TNPRC, which is undergoing a vigorous expansion, currently includes NIH-funded research programs on AIDS, Lyme disease, tuberculosis, varicella, malaria, and Category A-C Select Agents, using chiefly nonhuman primates but also other animal models. The successful candidate will be expected to contribute to existing research programs and to build or bring his/her own independent research agenda. All necessary resources to assure that the candidate is successful will be provided including ample laboratory and office space.

The TNPRC has excellent infrastructure to support collaborative and independent research using nonhuman primates. In addition to holding one of the largest colonies of nonhuman primates in the country, the TNPRC is the only National Primate Research Center that houses a Regional Biosafety Laboratory, to support research under the NIH/NIAID National Biodefense Program. Research resources include extensive BSL2/ABSL2 and BSL3/ABSL3 facilities and highly integrated clinical and laboratory support for infectious disease studies using nonhuman primates. This includes a full-time staff of clinical veterinarians and technicians and core services commonly used for infectious disease research including: (1) Diagnostic Parasitology, (2) Vector-Borne Diseases (maintains ixodid ticks and anopheline mosquitoes), (3) DNA Microarray and Gene Expression, (4) Anatomic Pathology, (5) Clinical Pathology, (6) Molecular Pathology, (7) Confocal Microscopy and Image Analysis, (8) Flow Cytometry, (9) Cellular Immunology, (10) Virus Characterization, Isolation and Production, (11) Pathogen Detection and Quantification, and (12) Infectious Disease Aerobiology. More information is available at the following link: [http://www.tnprc.tulane.edu/research\\_resou.html](http://www.tnprc.tulane.edu/research_resou.html).

To apply, send a letter indicating your research interests and experience, curriculum vitae, and the names of three individuals who may be contacted for references to: **Ms. Rita Haynes, Coordinator, TNPRC Search Committee, Tulane National Primate Research Center, 18703 Three Rivers Road, Covington, LA 70433; E-mail: [rita@tulane.edu](mailto:rita@tulane.edu).**

*Tulane University is an Affirmative Action and Equal Opportunity Educator and Employer. Women and minorities are strongly encouraged to apply.*

# GRANTS FOR BIOMEDICAL RESEARCH INTERNATIONAL EARLY CAREER SCIENTISTS

Science is a multinational, cross-cultural endeavor that connects researchers across the borders created by discipline and continent. The Howard Hughes Medical Institute (HHMI) believes it is vital to encourage the careers and scientific creativity of scientists working abroad. HHMI is announcing an International Early Career Scientist Program, which will support up to 35 outstanding scientists working in selected countries outside the United States who are, or have the potential to become, scientific leaders.

## Five-year basic biomedical research grant

**Application deadline:**  
**February 23, 2011,**  
**at 2 p.m. ET, U.S.**

**Application information:**  
<http://www.hhmi.org/research/competitions>

The International Early Career Scientist Program will select and support highly qualified scientists working in selected countries outside the United States who are in the critical beginning stages of their independent careers. HHMI international early career scientists will receive five-year grants—\$250,000 in the first year and \$100,000 for each of the following four years.

Successful applicants will have trained through the postdoctoral level in a vigorous basic research environment. Eligible candidates must have trained in the United States at the doctoral, medical, or postdoctoral level. Applicants are expected to have outstanding scientific training records and exceptional potential for significant productivity and originality in their independent careers.

HHMI recognizes that a supportive research environment is crucial to launching a successful research program, so awards will be made only to institutions that can clearly support the research activities of the grant recipient.

Applicants must meet the following eligibility requirements:

- Hold a doctoral degree or medical degree and have completed postdoctoral research training.

- Have trained at the doctoral, medical, or postdoctoral level in the United States.
- Hold a full-time position as an independent researcher at a research-oriented university, medical school, or nonprofit institution in any of the following countries: Argentina, Brazil, Chile, China, Czech Republic, Egypt, Hungary, India, Italy, Mexico, Poland, Portugal, Russia, South Africa, South Korea, Spain, Taiwan, Turkey.
- Have made significant contributions in fundamental biomedical research on basic biological processes or disease mechanisms, and have been the first or senior author on at least two peer-reviewed, English-language, original research publications.
- Have started their first independent research position on or after January 1, 2004.
- Control their own research direction, laboratory space, and funding and devote most of their professional time to research, mentoring, and teaching.

*HHMI, a nonprofit medical research organization, plays a powerful role in advancing biomedical research and science education. To learn more, visit [www.hhmi.org](http://www.hhmi.org).*





**Assistant/Associate/Full Professor  
Faculty Position in Infectious Diseases Research  
Tenure/Tenure Track Faculty, 9-Month Appointment  
(80% Research and 20% Teaching)  
Position Number 103761**

The Virginia-Maryland College of Veterinary Medicine at the University of Maryland-College Park, MD, invites applications from the qualified individuals for a tenure/tenure-track faculty position in Immunology of Infectious Diseases and Microbial Pathogenesis. This 9-month appointment will be at the Assistant, Associate or Full Professor level, depending on the qualifications of the selected candidate. The Department has an excellent core facility, which includes BSL-2 and BSL-3 suites.

**QUALIFICATIONS:** A DVM/PhD or PhD degree with relevant postdoctoral training.

**RESEARCH:** Current focus is on host-pathogen interaction with emphases on virology, immunology, microbial pathogenesis, epidemiology, and public health. The position requires the candidate to focus on host immunity/host-pathogen interactions with importance for human and animal health, zoonosis and public health. The selected candidate will be expected to develop, maintain, and conduct a productive, extramurally funded research program that will strengthen the current research goals of the College. Applicants at the Associate/Full Professor level are expected to have an established, extramurally-funded research program and a strong publication record. The selected candidate will also be expected to develop and maintain active and productive collaborations, both within and outside the College. Excellent opportunities exist for collaboration with federal agencies (USDA, FDA, NIH) and other University departments.

**TEACHING:** Active participation in the University's Graduate Program will be required, to include mentoring of graduate students and serving on student advisory committees. The selected candidate will be responsible for the development of one graduate course to be included in the current veterinary medicine curriculum. This new course will be developed in conjunction with active participation on the college's current teaching objectives and mission goals. Salary will be commensurate with rank and experience.

**TO APPLY:** Applications are accepted through <https://jobs.umd.edu> (sort for position number 103761). Required are (1) cover letter, (2) CV, (3) statement of research interests and plans, (4) statement of teaching philosophy and (5) three reference letters. For best consideration, applications should be received by **January 31, 2011**, or until a suitable candidate is identified.

*THE UNIVERSITY OF MARYLAND IS AN AFFIRMATIVE ACTION/EQUAL OPPORTUNITY  
EMPLOYER WOMEN AND MINORITIES ARE ENCOURAGED TO APPLY.*

In 2011,  
**CNRS**  
is recruiting  
377 permanent  
researchers  
in all scientific fields

- LIFE SCIENCES
- CHEMISTRY
- ENVIRONMENTAL SCIENCES  
AND SUSTAINABLE  
DEVELOPMENT
- HUMANITIES AND SOCIAL  
SCIENCES
- COMPUTER SCIENCE
- ENGINEERING
- MATHEMATICS
- PHYSICS
- NUCLEAR AND PARTICLE  
PHYSICS
- EARTH SCIENCES AND  
ASTRONOMY

Online registration at [www.cnrs.fr](http://www.cnrs.fr)  
from December 1, 2010  
Registration deadline  
January 6, 2011



**Full Professors/Group Leaders  
Available at the State Key Laboratory  
of Ophthalmology in China**

Established in 1965, Zhongshan Ophthalmic Center of Sun Yat-sen University is a leading eye care institution in China. It comprises the Eye Hospital, the Eye Research Institute, the Department of Preventive Ophthalmology and the Department of Optometry.

The China State Key Laboratory of Ophthalmology at the Zhongshan Ophthalmic Center seeks full professors or group leaders. Candidates should have a strong academic record in eye and vision research. Scientific excellence and an interactive personality are the most important criteria for selection. Non-Chinese scientists are also encouraged to apply. All positions are full-time and based in Guangzhou, China.

Applicants should submit a *curriculum vitae*, a letter explaining their research plan in the position and an indication of their salary expectation to:

The Searching Committee  
Zhongshan Ophthalmic Center  
54 South Xianlie Road  
Guangzhou, 510060  
China

Fax +8620-87333271  
[ZOC@mail.syu.edu.cn](mailto:ZOC@mail.syu.edu.cn)  
[www.gzoc.com](http://www.gzoc.com)

**The close date is December 31, 2010.**



**Do you have a passion for cutting-edge science and educating the public about it?**

**Five Chief Scientists for the Nature Research Center (NRC), North Carolina Museum of Natural Sciences**

The North Carolina Museum of Natural Sciences in Raleigh, NC will open an 80,000 square-foot innovative facility in early 2012 to serve as a public laboratory for both local and global science research ([www.naturesearch.org](http://www.naturesearch.org)). The NRC seeks five Science Directors to lead its new research laboratories: Genetics/Microbiology; Paleontology/GeoSciences; Space Observation; Biodiversity/Earth Observation; and Science Communication. Over 40 additional staff will be hired to operate four research-and-education platforms: (1) A three-story theater, Daily Planet, with the latest technologies for communicating science; (2) Investigate Labs for citizen science participation; (3) Exhibits featuring staff research and other critical global issues; and (4) Hands-On Programs of citizen science/distance learning/mentoring/outreach to diverse audiences including policy-makers. Directors will execute cutting-edge research programs under the leadership of Dr. Meg Lowman, Director of the NRC, as well as lead a team of lab managers, education staff, students, and citizen scientists. In addition, Directors will have joint appointments at the level of Associate or Full Professor with one of the 16 campuses of the UNC System, offering additional research status and some teaching opportunities. Salary is funded between University and Museum, with a competitive benefits package.

Applicants who enthusiastically embrace education outreach as part of their outstanding research programs are invited to submit a letter of interest by December 30, 2010. Suitable candidates must have a PhD or equivalent in fields relevant to one of the research labs, 3-5 years postdoctoral or research/teaching/ administrative experience, and proven expertise with science communication to diverse audiences. The application process requires two stages: (1) Interest Period and (2) Application Period. During the interest period please send a letter of interest, if you choose you can also send, CV; 3 research publications (including one oriented to public audiences); 3 letters of reference plus 3-5 additional contacts; and a vision statement outlining an unsolved issue in your scientific arena, and how you could conduct research/communicate outreach in the NRC facilities. Please mail to: **Deputy Museum Director, Alvin Braswell, NC Museum of Natural Sciences, 11 W Jones St, Raleigh NC 27601; or email to: Cindy Bogan, administrative assistant, [cindy.bogan@ncdenr.gov](mailto:cindy.bogan@ncdenr.gov) (919-733-7450 x 262).**

Subsequent to submitting the letter of interest, interested persons will be notified when to submit a state application form (PD-107) listing the position number and job title, relevant information/work history by 5 PM on the due date listed on the state website. Please note all relevant knowledge, skills, abilities, training and experience must be documented on the PD-107 state application form to receive proper credit and consideration (<http://www.osp.state.nc.us/jobs/gnrinfo.htm>).

*NCMNS is an Equal Opportunity/Affirmative Action Employer.*

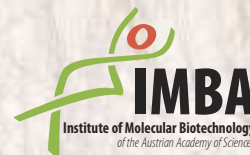
# GROUP LEADER POSITIONS at IMBA, Vienna

We invite applications for two fully funded groupleader positions at IMBA, Vienna. We aim to identify outstanding candidates who will establish a strong and innovative research program in any area of cell or molecular biology or molecular medicine.

We offer internationally competitive salaries, generous funding for up to four positions including all set up and running costs. We provide free access to our core service infrastructure including proteomics, Drosophila facility, FACS sorting, deep sequencing, bio-optics and a 2000m<sup>2</sup> mouse house. PhD students are recruited through the Vienna Biocenter PhD program, one of the most competitive graduate programs in Europe. Child care facilities are available.

IMBA is an internationally recognized research institute that focuses on stem cell research, RNA biology and molecular medicine and is home to 140 scientists of over 26 nationalities. Together with the neighbouring IMP and the Max Perutz laboratories, we provide a dynamic and highly interactive research environment.

Applications including CV, scientific achievements, a five page future research plan and contact details for three referees should be sent electronically to [tlsearch@imba.oew.ac.at](mailto:tlsearch@imba.oew.ac.at) by January 31st, 2011. For further information on IMBA see: [www.imba.oew.ac.at](http://www.imba.oew.ac.at)



## POSTECH POHANG UNIVERSITY OF SCIENCE AND TECHNOLOGY

### UNIVERSITY PROFESSOR POSITIONS

The Pohang University of Science and Technology (POSTECH) is Korea's first research-oriented university and is known for its unique contribution to the advancement of science and technology in Korea. POSTECH is ranked first among Asia's specialized universities in the 2010 Chosun Ilbo - QS Asian University Rankings.

**POSTECH invites distinguished scholars to join in POSTECH's efforts to become a global leader in science & technology research.**

#### Position Announcement:

We are seeking distinguished scholars in all fields of science and engineering. Appointments will be made for multiple positions at the rank of University Professor. POSTECH will provide startup funds of up to 7 million US dollars for each distinguished scholar. Salary will be competitive and commensurate with candidate's qualifications and experience. The exact amount of financial support may vary depending on the field.

#### Desired Qualifications:

- Nobel laureates, Fields medalists or scholars of equivalent caliber
- Members of world-renowned national academies of sciences/engineering such as the National Academy of Sciences (USA), the National Academy of Engineering (USA), and the Royal Society (UK)
- Editors of the world's top scientific journals or those with equivalent experience
- Those who have demonstrated potential to grow to become a University Professor are also welcome to apply.

Interested individuals should send a CV, a statement of research plans, and a teaching statement to **e-mail: [recruit@postech.ac.kr](mailto:recruit@postech.ac.kr)** or **mailing address: Faculty Search Committee, Office of Academic Affairs, POSTECH, San 31, Hyoja-dong, Nam-gu, Pohang 790-784, Republic of Korea.**





Office of the Science and  
Technology Adviser to the  
Secretary of State

## Jefferson Science Fellowship

The National Academies is pleased to announce a call for applications for the 2011 Jefferson Science Fellows program. This program is a model for engaging the American academic science, technology and engineering communities in the formulation and implementation of U.S. foreign policy.

Jefferson Science Fellows spend one year at the U.S. Department of State or the U.S. Agency for International Development in Washington, DC and may periodically travel to U.S. foreign embassies and/or missions.

Jefferson Science Fellow awards are open to tenured academic scientists, technologists and engineers from U.S. institutions of higher learning. Applicants must be U.S. citizens and will be required to obtain a security clearance. Detailed information on the Jefferson Science Fellows program is available online:

**[www.nas.edu/jsf](http://www.nas.edu/jsf)**

The deadline for applications for the 2011 program year is  
**January 14, 2011.**

The Jefferson Science Fellows program is sponsored by the U.S. Department of State and U.S. Agency for International Development.

**THE NATIONAL ACADEMIES**  
*Advisers to the Nation on Science, Engineering, and Medicine*



## Promega is Hiring Due to Growth in Research & Applied Markets

With strong corporate growth and double digit investment in R&D, Promega continues to expand its scientific staff to develop new technological capabilities for the life sciences.

Promega is seeking additional scientific expertise across multiple areas, including **analytical methods in cellular biology and proteomics, nucleic acid isolation and analysis, high throughput assays, laboratory automation, toxicology, and human identity.**

Requirements include:

- Desire to innovate new tools for advancing basic research, drug discovery, human identity, and molecular diagnostics
- Willingness to work in a dynamic team environment engaged in diverse disciplines.
- Interest in sharing scientific achievements and collaborating with innovators from other companies, universities, and institutes.

Promega is located in Madison, WI, a community of international scientists, beautiful natural settings and an award winning quality of life.

Established in 1978, Promega is a privately held company supplying 2000+ products to over 100 countries.

**Visit [www.promega.com/RDjobs](http://www.promega.com/RDjobs)**

Promega Corporation is an equal opportunity/affirmative action employer.



**CBG**  
Max Planck Institute  
of Molecular Cell Biology  
and Genetics

The Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) in Dresden is seeking outstanding candidates for

## Research Group Leader in Membrane Biology

Research at the MPI-CBG focuses on the molecular mechanisms underlying the structure and organization of cells and tissues (see <http://www.mpi-cbg.de>). In this search, we are particularly looking for applicants interested in membrane biochemistry, though all excellent candidates will be considered.

Research Group Leader positions are initially for 5 years at the EG15 level according to the TVÖD scale with the possibility of extension for up to an additional 4 years. Funds are available for a postdoctoral fellow, a PhD student, a technician, as well as for consumables and equipment. Please send your CV, publication list and a short description of research accomplishments and future plans to Prof. Marino Zerial (see address below) by 7 January 2011. Please also arrange for three letters of reference to be sent separately before the deadline. We especially encourage women to apply.

**Prof. Marino Zerial**  
Max Planck Institute  
of Molecular Cell Biology and Genetics  
Pfotenhauerstr. 108  
01307 Dresden, Germany



Please send your applications to: [2010-RGL-1080@mpi-cbg.de](mailto:2010-RGL-1080@mpi-cbg.de)

The Max Planck Society is committed to employ more handicapped individuals and actively seek their applications.



**Chair, Department of Biological Sciences**  
**Pocatello, Idaho**

**Department:** Biological Sciences

**Primary Purpose:** Serve as a faculty member and Chair of Biological Sciences.

**Minimum Qualifications:** Doctoral Degree and credentials to qualify for tenure and appointment at the level of full professor.

**Preferred Qualifications:** The successful applicant will have a Doctorate in biological sciences or related field, a commitment to undergraduate and graduate education, an excellent record of publication and extramural support, and strong leadership qualities; candidate should have a proven track record in research.

**Salary/Pay Information:** Commensurate with qualifications and experience. Competitive benefits package.

**Term of Employment:** 12 Month /Full-time

**Location:** Pocatello

**Application Process:** Please visit ISU at  
[https://isujobs.net/applicants/jsp/shared/Welcome\\_css.jsp](https://isujobs.net/applicants/jsp/shared/Welcome_css.jsp)

**Special Instructions to Applicants:** Please include in your cover letter a statement of leadership philosophy.

**Closing Date:** Open Until Filled

ISU is an equal opportunity/affirmative action employer. We have an institution-wide commitment to inclusion and diversity and encourage all qualified individuals to apply. Veterans' preference. Upon request, reasonable accommodations in the application process will be provided to individuals with disabilities.





Faculty of Science

The Faculty of Science invites applications for an

## Assistant Professorship in Physical Chemistry

in the area of the structure and dynamics of molecular systems in the condensed phase (experimental or theoretical). The new professor is expected to pursue cutting-edge research in an area that is complementary to that of existing groups at the University of Zurich. He or she is furthermore expected to teach special courses in Chemistry at the Master or PhD level in either German or English.

The Institute of Physical Chemistry is located on the Irchel campus, where most of natural science Institutes of the University are located. The Faculty of Science provides for a very stimulating and attractive environment for interdisciplinary research, which is further strengthened by the nearby science campus of the ETH Zurich, the Empa and the Paul Scherrer Institute.

The position will be filled at the Assistant Professor level (non-tenure track, for six years). Candidates are invited to submit an application package by January 31, 2011, including curriculum vitae, publication list, outline of current and future research interests, teaching philosophy and names and addresses of three potential referees. Documents should be addressed to Prof. Dr. Michael Hengartner, Dean of the Faculty of Science, University of Zurich, and submitted as a single PDF file to [jobs@mnf.uzh.ch](mailto:jobs@mnf.uzh.ch). For further information, please contact Prof. Dr. P. Hamm at [phamm@pci.uzh.ch](mailto:phamm@pci.uzh.ch)

The University of Zurich is an equal opportunity employer. Applications from women are particularly encouraged.



Eidgenössische Technische Hochschule Zürich  
Swiss Federal Institute of Technology Zurich

## Assistant Professor (Tenure Track) of Cartilage Engineering and Regeneration

The future Department of Health Sciences and Technology at ETH Zurich invites applications for the above mentioned position. The new professor is expected to bridge the gap between the fundamental biosciences and clinical applications by working out an outstanding research program, developing innovative methods that promote cartilage regeneration. Key components of this research program could center around the development of new insights into the molecular structure of cartilage matrix proteins, cartilage signaling, mechanobiological aspects, mechanisms regulating cartilage metabolism, as well as repair and regeneration mechanisms. These insights would be utilized to define novel tissue engineering strategies to replace or regenerate cartilage (both articular and septal) and joint tissues (i.e. meniscus and synovium) that interact with cartilage. The primary criterion is either demonstrated or potential excellence in research and technology developments, rather than on one particular scientific aspect. The professorship comes with generous resources, including start-up funds and annual allocations, to establish a significant research program. Close collaboration with researchers at ETH Zurich, the FIFA Medical Assessment Research Center/Schulthess Clinic and University Hospital Zurich are envisioned, thereby complementing already existing efforts.

The candidate will contribute to the interdisciplinary education programs offered at ETH Zurich; this includes the ETH Health Sciences and Technology Bachelor and Master Program, and the ETH Master Program in Biomedical Engineering. The new professor will be expected to teach undergraduate level courses (German or English) and graduate level courses (English). The candidate's educational program will support a vital link between biological and physical scientists and engineers and clinical professionals.

Assistant professorships have been established to promote the careers of younger scientists. The initial appointment is for four years with the possibility of renewal for an additional two-year period and promotion to a permanent position.

Please submit your application together with a curriculum vitae, a list of publications and a brief statement of present and future research interests **to the President of ETH Zurich, Prof. Dr. Ralph Eichler, ETH Zurich, Raemistrasse 101, 8092 Zurich, Switzerland (or via e-mail as one single PDF to [faculty-recruiting@sl.ethz.ch](mailto:faculty-recruiting@sl.ethz.ch)), no later than January 31, 2011.** With a view towards increasing the number of female professors, ETH Zurich specifically encourages qualified female candidates to apply.





### Department of Environmental Health Sciences, Professor and Chair

The Johns Hopkins Bloomberg School of Public Health invites applications for chair of the Department of Environmental Health Sciences. The successful applicant will have an outstanding record of academic and research accomplishment, and demonstrated leadership and administrative abilities.

The Department integrates diverse disciplines in its quest to increase understanding of the impact of environmental factors on individuals and human populations. It has over 60 faculty, 80 Doctoral and Master's students, and a diverse research program. The Bloomberg School of Public Health has ten departments and more than 500 full-time faculty and 2000 graduate-level students. The School is located on the Johns Hopkins University East Baltimore medical campus, a collaborative and highly interactive environment with a superb research infrastructure.

The Johns Hopkins University is committed to recruiting, supporting and fostering a diverse community of outstanding faculty, staff and students. All applicants who share this goal are encouraged to apply. Women and underrepresented minority candidates are particularly encouraged to apply. The Johns Hopkins University is an Equal Opportunity Employer and does not discriminate on the basis of race, color, gender, religion, age, sexual orientation, national or ethnic origin, disability, marital status, veteran status, or any other occupationally irrelevant criteria. The University promotes affirmative action for minorities, women, disabled persons, and veterans.

Applications should include a curriculum vitae and statement of research interest and vision of leadership. Please submit electronic applications as PDF or doc files. Review of candidates will begin in **January 2011**. Applications should be submitted to:

**Chair, Search Committee for  
Chair of Environmental Health Sciences  
c/o Ms. Susan Williams, Special Assistant  
Office of the Dean  
Johns Hopkins Bloomberg School of Public Health  
615 N. Wolfe St., Room W1041  
Baltimore, MD 21205  
suwillia@jhsph.edu**



The Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) and the Max Planck Institute for the Physics of Complex Systems (MPI-PKS), both located in Dresden, are seeking outstanding candidates for

### Research Group Leaders in Systems Biology

The successful applicants could be theoretical, experimental or both, and are expected to develop an independent research program. Research at the MPI-CBG focuses on the structure and dynamic organization of cells and tissues in several model organisms (see <http://www.mpi-cbg.de>). This experimental research is greatly strengthened by theory, contributed through close collaborations with physicists at the MPI-PKS (<http://www.mpi-pks-dresden.mpg.de>).

In Dresden, we define systems biology broadly. In addition to more traditional activities in systems biology such as the analysis of genetic and protein signaling networks, we have a strong interest in the quantitative understanding of morphogenesis at several levels: how do molecules dynamically organize into organelles and cells, and how do interactions between cells give rise to tissues and organs? Answering these questions requires experimental and theoretical approaches, in which there is considerable strength in Dresden, but also advances in the processing and synthesis of biological data, especially images. We therefore seek applicants not just from biology, bioinformatics and physics, but also from computer science and engineering.

MPI Research Group Leader positions are initially for 5 years at the EG15 level according to the TVÖD scale with the possibility of an extension. Funds are available for personnel, consumables and equipment according to the procedures at the two Institutes. Please send your CV, publication list and a short description of research accomplishments and future plans to Prof. Marino Zerial or Prof. Roderich Moessner (see addresses below), mentioning Systems Biology search, by 7 January 2011. Please also arrange for three letters of reference to be sent before the deadline. We especially encourage women to apply.

**Prof. Marino Zerial**  
Max Planck Institute of Molecular Cell Biology and Genetics  
Pfotenhauerstr. 108, 01307 Dresden, Germany

**Prof. Roderich Moessner**  
Max Planck Institute for the Physics of Complex Systems  
Noethnitzer Str. 38, 01187 Dresden, Germany

Please send your applications to: [2010-RGL-1080@mpi-cbg.de](mailto:2010-RGL-1080@mpi-cbg.de)

The Max Planck Society is committed to employ more handicapped individuals and actively seek their applications.



### Faculty Position Opportunity

#### Division of Cancer Medicine, Department of Lymphoma/Myeloma

The University of Texas MD Anderson Cancer Center seeks an M.D. or Ph.D. scientist to join its full-time faculty. This opportunity provides dedicated research effort to actively engage in cutting-edge translational research. Multiple opportunities for collaboration with scientists within the institution and faculty mentorship. The selected applicant should have expertise in laboratory research. Faculty rank is Assistant Professor, tenure track. A generous start-up package is available.

MD Anderson, ranked as the top cancer center by *U.S. News & World Report*, is the world's largest treatment facility for oncological diseases. Our location in Houston provides access to a world-renowned medical community and the splendid cultural and recreational diversity of a sophisticated, metropolitan area that is the country's fourth largest city.

**Interested applicants should send a copy of their curriculum vitae to:**

**Larry W. Kwak, M.D., Ph.D.**  
**Professor and Chairman**  
**Justin Distinguished Chair in Leukemia Research**  
**Department of Lymphoma/Myeloma**  
**The University of Texas MD Anderson Cancer Center**  
**1515 Holcombe Blvd., Unit 429**  
**Houston, TX 77030**

THE UNIVERSITY OF TEXAS  
**MD Anderson**  
**Cancer Center**

Making Cancer History®

MD Anderson Cancer Center is an equal opportunity employer and does not discriminate on the basis of race, color, national origin, gender, sexual orientation, age, religion, disability or veteran status except where such distinction is required by law. All positions at The University of Texas MD Anderson Cancer Center are security sensitive and subject to examination of criminal history record information. Smoke-free and drug-free environment.

### Positions in Regenerative Biology and Tissue Engineering

The Marine Biological Laboratory (MBL) and the Harvard University Department of Stem Cell and Regenerative Biology announce a joint search for outstanding, highly innovative candidates at Assistant, Associate, and/or Senior levels in the recently established Eugene Bell Center for Regenerative Biology and Tissue Engineering at the MBL in Woods Hole, Massachusetts. Successful candidates are expected to develop independent research programs in the areas of stem cell and regenerative biology or tissue engineering, focusing in particular on marine, aquatic or lower vertebrate models.

Areas of emphasis include, but are not limited to, gene regulatory networks and systems analysis, genomics, comparative evolutionary biology, bioelectric currents, advanced imaging, and computational approaches. Exploration of collaborative opportunities and synergies with other research programs at both the MBL and Harvard is encouraged. Opportunities for teaching in undergraduate and graduate programs at Harvard University are provided through an affiliated faculty appointment in the Department of Stem Cell and Regenerative Biology.

Dr. Gary Borisy is the chair of the search committee.

**ADVISORY COMMITTEE:** Doug Melton, Chair, Harvard University; Alejandro Sanchez Alvarado, University of Utah; Eric Davidson, California Institute of Technology; Linda Griffith, Massachusetts Institute of Technology; Michael Levin, Tufts University; Elly Tanaka, Max Planck Institute, Dresden.

For applications and more information please visit:

**<https://mbl.simplehire.com>**

*The MBL is an Equal Opportunity/Affirmative Action workplace.*

# Discover the next breakthrough. Challenge the future of science.

## BE PART OF THE FOUNDING FACULTY OF SCIENTISTS AND ENGINEERS.

The Singapore University of Technology and Design (SUTD), established in collaboration with the Massachusetts Institute of Technology (MIT), is seeking exceptional faculty members for this new university slated to matriculate its first intake of students in April 2012.

SUTD, the first university in the world with a focus on design accomplished through an integrated multi-disciplinary curriculum, has a mission to advance knowledge and nurture technically grounded leaders and innovators to serve societal needs. SUTD is characterised by a breadth of intellectual perspectives (the "university"), a focus on engineering foundations ("technology") and an emphasis on innovation and creativity ("design"). The University's programmes are based on four pillars leading to separate degree programmes in Architecture and Sustainable Design, Engineering Product Development, Engineering Systems and Design, and Information Systems Technology and Design. Design, as an academic discipline, cuts across the curriculum and will be the framework for novel research and educational programmes.

MIT's multi-faceted collaboration with SUTD includes the development of new courses and curricula, assistance with the early deployment of courses in Singapore, assistance with faculty and student recruiting, mentoring, and career development, and collaborating on a major joint research projects, through a major new international design centre and student exchanges. Many of the newly hired SUTD faculty will spend up to year at MIT in a specially tailored programme for collaboration and professional development.

### FACULTY POSITIONS

The qualifications for the faculty position include: an earned doctorate in Biology, Biomedical Sciences, Chemistry, Chemical Engineering, a strong commitment to teaching at the undergraduate and graduate levels, a demonstrated record of, or potential for scholarly research, and excellent communication skills.

We invite applications for interdisciplinary faculty appointments at all levels, with many opportunities available in particular at the Assistant and Associate Professor levels. Duties include teaching of graduate and undergraduate students, research, supervision of student research, advising undergraduate student projects, and service to SUTD and the community. Faculty will be expected to develop and sustain a strong research programme. Attractive research grant opportunities are also available.

Successful candidates can look forward to internationally competitive remuneration, and assistance for relocation to Singapore. If you want to be part of the founding faculty that will shape the world of tomorrow, please apply to SUTD at [www.sutd.edu.sg](http://www.sutd.edu.sg)



PO Box 2800  
Pretoria 0001  
South Africa  
Tel: (012) 481 4000  
Fax: (012) 348 1179  
Int. Code: +27 12  
info@nrf.ac.za

[www.nrf.ac.za](http://www.nrf.ac.za)

### ADVERTISEMENT

#### DIRECTOR: SOUTH AFRICAN ASTRONOMICAL OBSERVATORY (SAAO)

The SAAO is a National Facility operated by the National Research Foundation of South Africa and is the primary facility for optical/infrared astronomy in Africa. It has a staff of about 120, divided between the headquarters in Cape Town, where the Director will be based, and the observing facilities at Sutherland in the Northern Cape.

The Southern African Large Telescope (SALT), the single largest optical telescope in the southern hemisphere, is operated by SAAO, under contract to the 12-member international SALT consortium. SAAO has a divisional structure that consists of (a) astronomy research, (b) SALT astronomy and technical operations, (c) small telescope operations, (d) instrumentation (which has been responsible for major components of two of the SALT primary instruments), (e) IT (recently upgraded through its location to a modern, dedicated building), (f) education and public outreach (through the SALT collateral benefits program, and recently enhanced through the IAU award to SAAO of its Office of Astronomy for Development), (g) administration.

We are seeking a dynamic astronomer with excellent leadership skills and diplomatic qualities, an established track record in optical/infrared astronomy, proven experience and skills in research management and a reputation for high quality research at an international level. Experience with large telescope science and/or instrumentation would be an advantage.

Further information about SAAO, SALT and the NRF may be obtained through <http://www.sao.ac.za>, together with a more detailed job description.

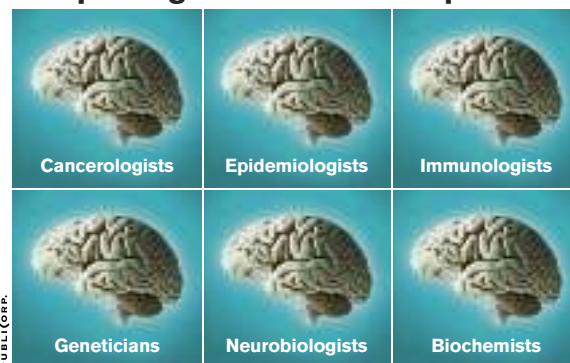
Applicants should submit a letter of application, motivating their candidacy for the position, together with a detailed CV, which includes the names, addresses and contact numbers of at least three referees, before 30 January 2011 to:

Mr Patrick Thompson  
Group Executive: HR and Stakeholder Relations  
Fax: +27 (0)12 481 4006 / E-mail: [Patrick@nrf.ac.za](mailto:Patrick@nrf.ac.za)



*The NRF is committed to employment equity and redress.*

## Improving human health requires...



## Inserm 122 tenure positions are offered to researchers m/f dedicated to biomedical research

Candidates to Research Associates and Research Directors positions must have a PhD (or equivalent degree). There is no nationality restriction.

Inserm is the only French public research body entirely dedicated to human health. Its researchers are committed to studying all diseases, whether common or rare.

Through its diversity of approaches, Inserm provides a unique environment for researchers.

13 000 researchers, engineers and technicians work in the 318 Inserm laboratories housed in hospitals, universities and research campuses, all over France.

**Application modalities:** visit our website : <http://www.eva2.inserm.fr>  
**Application deadline:** January 6th, 2011 - 4.00pm (GMT+1)



Institut national  
de la santé et de la recherche médicale





## Download your free copy today.

**ScienceCareers.org/booklets**

- **Business Sense:** Starting an Academic Lab
- **Lab Management:** The Human Elements
- **Mind Matters:** In Defense of Downtime
- **Funding Your Future:** Publish or Perish
- **If at First You Don't Succeed,** Cool Off, Revise, and Submit Again
- **Your Research in the Headlines:** Dealing with the Media

**Science Careers**

From the journal *Science*



Brought to you by the AAAS/Science Business Office

# VCU

## TENURE-TRACK/TENURED FACULTY POSITIONS DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY Virginia Commonwealth University School of Medicine

We are seeking exceptional investigators pursuing innovative questions relevant to the department's multi-disciplinary research strengths in cellular and molecular biology, signaling, metabolism, inflammation, and cancer. Candidates should have a PhD or MD/PhD with an outstanding research record and will be considered at all ranks based upon qualifications and experience. A strong record of research accomplishments, an independent externally funded research program, and experience working in and fostering a diverse faculty, staff, and student environment or commitment to do so as a faculty member at VCU are required. A leadership position is available for an outstanding senior individual. Substantial startup and salary packages are available. The Department belongs to a very active community of investigators and is committed to providing an outstanding research environment.

Interested candidates should send curriculum vitae, letter of interest including research outline and the name, address, telephone number, fax number, and e-mail address of three references. Electronic submissions preferred to: [mwmaceyka@vcu.edu](mailto:mwmaceyka@vcu.edu). Mailing address: Dr. Sarah Spiegel, Chair, Virginia Commonwealth University, Department of Biochemistry and Molecular Biology, Box 980614, Richmond VA 23298-0614.

*Virginia Commonwealth University is an Equal Opportunity/  
Affirmative Action Employer. Women, minorities and persons  
with disabilities are encouraged to apply.*



**CBG**  
Max Planck Institute  
of Molecular Cell Biology  
and Genetics

The Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) in Dresden is seeking outstanding candidates for

## Research Group Leader in Electron Microscopy

Research at the MPI-CBG focuses on the molecular mechanisms underlying the structure and organization of cells and tissues (see <http://www.mpi-cbg.de>). In this search, we are particularly looking for applicants using electron microscopy, including cryotomography, to approach these issues, though all excellent candidates will be considered.

Research Group Leader positions are initially for 5 years at the EG15 level according to the TVÖD scale with the possibility of extension for up to an additional 4 years. Funds are available for a postdoctoral fellow, a PhD student, a technician, as well as for consumables and equipment. Please send your CV, publication list and a short description of research accomplishments and future plans to Prof. Marino Zerial (see address below) by 7 January 2011. Please also arrange for three letters of reference to be sent separately before the deadline. We especially encourage women to apply.

**Prof. Marino Zerial**  
Max Planck Institute  
of Molecular Cell Biology and Genetics  
Pfotenhauerstr. 108  
01307 Dresden, Germany



Please send your applications to: [2010-RGL-1080@mpi-cbg.de](mailto:2010-RGL-1080@mpi-cbg.de)

The Max Planck Society is committed to employ more handicapped individuals and actively seek their applications.



**Department of Health and Human Services  
National Institutes of Health**



**Clinical Director  
National Institute of Arthritis and Musculoskeletal and Skin Diseases**

The Intramural Research Program of the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) is seeking a physician-scientist to serve as Clinical Director. This individual will direct the NIAMS Program in Translational Research, which includes training and clinical care branches as well as multiple investigative laboratories and branches. Investigators in the Clinical Program conduct studies in natural history and treatment as well as basic investigations into the etiology and/or pathophysiology of disease. The candidate should have the ability to manage this diverse clinical research enterprise and to provide the leadership to maintain the outstanding track record of the NIAMS Clinical Program. The ideal candidate for this position is an M.D. or M.D.-Ph.D. who is board-certified or board-eligible in either Pediatrics, Internal Medicine and Rheumatology or Allergy/Immunology. Potential areas of concentration would include rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, vasculitis, scleroderma, myositis, osteoarthritis, or other inflammatory/rheumatic diseases. The candidate should have experience in conducting clinical or translational research and in immunology, cell biology, genetics, or other areas of research relevant to rheumatic or autoimmune disease. The candidate will also be provided generous independent resources to develop his/her own clinical or translational research program as a tenured investigator within NIAMS.

The NIAMS Clinical Program is part of the NIH Intramural Research Program, located in Bethesda, Maryland. Investigators in this program have full privileges to admit their research subjects, free of charge to the patient, to the new Mark O. Hatfield Clinical Research Center, a state-of-the-art 230-bed hospital fully devoted to clinical and translational studies. Ample resources will be provided to the successful applicant to establish a first-rate Clinical Program and their own research program in his or her area of concentration. The NIAMS Intramural Research Program, headed by its Scientific Director, Dr. John O'Shea, comprises several outstanding programs in cytokine biology, signaling, genetics, and structural biology, and the broader NIH Bethesda campus provides a rich and highly interactive environment within a wide range of basic and translational research disciplines that relate directly to rheumatology.

Salary will be commensurate with experience. A full package of benefits, including retirement, health, life, and long-term care insurance, and a Thrift Savings Plan, is available. Qualified international applicants who have passed the ECFMG or USMLE examinations or have comparable qualifications are welcome to apply.

Interested applicants should send a curriculum vitae, a one-page summary of research interests, and the names of three referees to **Ms. Linda Peterson, 31 Center Drive, MSC 2350, Building 31, Room 4C12, Bethesda, MD 20892-2350, e-mail [petersonl@arb.niams.nih.gov](mailto:petersonl@arb.niams.nih.gov). If you need additional information, please call Dr. John O'Shea at 301-496-2612.** Review of applications will begin on or about **January 3, 2011**, but applications will be accepted until the position is filled.

**NIH and DHHS are equal opportunity employers.**



**Science Careers** is the lens that magnifies opportunities.



Visit our **ENHANCED WEBSITE!**

Magnifying your opportunities is our main focus. Whether you're seeking a new job or career advancement in your chosen field, Science Careers will broaden your scope for a brighter future.

*Your Future Awaits.*

**Improved Website Features:**

- » New design for easier navigation
- » More relevant job search results
- » Automated tools for a more effective search

**Science Careers**  
From the Journal Science AAAS  
[ScienceCareers.org](http://ScienceCareers.org)

## UCRIVERSIDE

### FACULTY POSITIONS Department of Bioengineering Bourns College of Engineering

The Department of Bioengineering at the University of California, Riverside invites applications for junior level tenure-track faculty positions in the areas of translational technologies, including biomaterials, medical devices, diagnostic instrumentation, drug design and delivery, and/or the area of healthcare informatics. Exceptional senior candidates may be considered. Applicants should have a doctoral degree in the relevant engineering discipline or a related field.

Details and application materials can be found at [www.engr.ucr.edu/facultysearch](http://www.engr.ucr.edu/facultysearch). The search committee will review applications beginning on **January 25, 2011** and will continue to receive applications until the positions are filled.

*EEO/AA Employer.*



The University of New Mexico

### CELL/MOLECULAR BIOLOGIST Assistant Professor Department of Biology

The Biology Department at the University of New Mexico is seeking applications for a probationary appointment leading to a tenure decision Assistant Professor in the area of Cell/Molecular Biology. We seek a colleague who will establish and maintain a vigorous, independent research program, is committed to excellence in teaching at the undergraduate through graduate levels, and is interested in joining a broadly based Biology Department. Applicants must have a Ph.D. in biology or a related discipline and at least two years relevant postdoctoral experience. For complete job requirements see Assistant Professor, Cell Molecular Biologist at <https://unmjobs.unm.edu>.

Applicants must submit a cover letter, curriculum vitae, three representative reprints, and statements of current and future research and teaching interests. All application material must be uploaded and submitted through UNMjobs, <https://unmjobs.unm.edu>. Applicants must also arrange for at least three letters of reference to be sent directly to [Cellbios@unm.edu](mailto:Cellbios@unm.edu). Application materials must be received by **December 15, 2010**, for best consideration. Questions related to this posting may be directed to **Dr. Mary Anne Nelson**, [manelson@unm.edu](mailto:manelson@unm.edu).

*The University of New Mexico is an Equal Opportunity/Affirmative Action Employer and Educator. Women and underrepresented minorities are encouraged to apply.*



## Nontraditional Careers: Opportunities Away From the Bench Webinar

**Now Available  
On Demand**  
[www.sciencecareers.org/webinar](http://www.sciencecareers.org/webinar)

Produced by the  
Science/AAAS Business Office.





### Neurobiology Faculty Position

The Department of Anatomy and Neurobiology (<http://neurobiology.umaryland.edu>) is recruiting for tenured/tenure-track faculty positions in Neuroscience. We are particularly interested in candidates who will strongly complement existing strengths in the Department: chemical senses, peptidergic circuits, sensorimotor functions, neurodegeneration and neural circuits subserving higher order cortical functions. Candidates should have a strong history of scholarly activity and preference will be given to those with an independent funded research program and whose presence will catalyze multi-PI initiatives within the department.

We offer an outstanding intellectual and collaborative environment with highly competitive salary and recruitment packages. All department faculty are members of the Graduate Program in Life Sciences and the interdisciplinary Program in Neuroscience (<http://neuroscience.umaryland.edu>).

Candidates should submit the following as one single PDF file to [facsearch@umaryland.edu](mailto:facsearch@umaryland.edu): detailed curriculum vitae, a brief statement of research interests and goals, and names/contact information for three references. For best consideration candidates should submit their application by **February 1, 2011** and should be addressed to the attention of: **Dr. Joseph Cheer, Chair of Faculty Search Committee.**

*University of Maryland, Baltimore is an Equal Opportunity, Affirmative Action Employer. Minorities, women, veterans, and individuals with disabilities are encouraged to apply.*

## UAB THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

### UAB Stem Cell Institute Department of Biochemistry and Molecular Genetics University of Alabama at Birmingham Schools of Medicine and Dentistry

Tenure track junior faculty positions and tenured senior faculty positions are available for investigators focused on stem cell biology, biochemistry, epigenetics and transplantation biology. Areas of special emphasis include, but are not limited to, mechanistic studies of stem cell self-renewal and lineage specification and mechanisms of somatic cell reprogramming to pluripotency. Structural biology of stem cell proteins by X-ray crystallography and high-field NMR is an additional area of interest. State of the art X-ray crystallography instrumentation and a new 800MHz NMR system with a cryoprobe are available for Departmental faculty and Institute members. Nationally competitive salaries, startup packages and space allocations will be offered to successful candidates. UAB is a highly interactive environment with strong basic and clinical sciences. Birmingham is a beautiful and affordable city with many cultural attractions.

Applicants should send a C.V., a summary of research interests and the names of three references before **January 31, 2011** to:

**Dr. Tim Townes**  
**Director, UAB Stem Cell Institute**  
**Chairman, Department of Biochemistry and Molecular Genetics**  
**University of Alabama at Birmingham**  
**Kaul Genetics Building, Room 502**  
**720 20<sup>th</sup> Street South**  
**Birmingham, AL 35294**  
**E-mail: [ttownes@uab.edu](mailto:ttownes@uab.edu)**

*UAB is an Equal Opportunity Employer committed to building a culturally diverse environment.*



University Health Network  
Transforming Health Through Research and Innovation

### Tator-Campeau Endowed Chair in Brain and Spinal Cord Injury Research

The **Toronto Western Research Institute of the University Health Network** invites applicants to apply for a Scientist position. The ideal candidate will hold a PhD and/or MD degree (or equivalent) and have an established record of achievement in areas that have relevance to spinal cord injury or neurotrauma, e.g. neuroprotection, neuroregeneration and related disciplines such as neural stem biology, axon regeneration, neural plasticity or restorative neural engineering. The successful candidate will be appointed to an appropriate department at the University of Toronto at a level commensurate with their experience (from Assistant to Full Professor).

The UHN has a strong interdisciplinary research program in spinal cord injury and regenerative neuroscience. The successful candidate will have the ability to establish an independent, well funded program of international prominence and to collaborate with other members of our research and clinical staff in the development of treatments for injuries and disorders of the central nervous system.

**Applications (CV, statement of interest, referees) must be e-mailed by January 30, 2011 to: Dr. C.J. Paige, Vice President, Research, University Health Network, E-mail: [TCSearch@uhnresearch.ca](mailto:TCSearch@uhnresearch.ca).**

*We wish to thank all applicants for their interest, however, only those selected for an interview will be contacted. The Toronto Western Research Institute of the Toronto Western Hospital, along with the Princess Margaret Hospital and the Toronto General Hospital, is a member of the University Health Network, an equal opportunity Employer. In accordance with Canadian Immigration requirements, this advertisement is initially directed to Canadian citizens and permanent residents.*

## Guide the next generation of scientists.



### Dean, Graduate School of Biomedical Sciences

Reporting directly to the Executive Vice President for Academic and Clinical Affairs, you will be a key member of our leadership team. As chief academic and administrative officer of the Graduate School of Biomedical Sciences (GSBS) your primary responsibility will be to oversee the planning, development, and conduct of the academic programs of the school. Your interpersonal skills will serve you well as you represent GSBS in dealing with other schools within UMDNJ and with external academic, civic, governmental, and professional organizations. You will be proficient in fundraising and an innovative leader who can advance graduate training to meet the future needs of the biomedical and health sciences workforce.

The inspired candidate we seek must have a Ph.D., M.D. or M.D./Ph.D. degree, along with professional accomplishments that qualify for a Professorship with tenure. Previous experience as a Dean, Vice-Dean, Senior Associate Dean or Department Chair in an academic health sciences/research institution is preferred, as is a strong record of NIH funded research. A successful history of graduate student mentoring and program development, together with the skills to engender collaboration among groups of diverse backgrounds, are also essential.

For consideration, please send your letter of interest and curriculum vitae to: **UMDNJ-GSBS Dean Search Committee, c/o P.R. Falk, Office of Academic Affairs, University of Medicine and Dentistry of New Jersey, 65 Bergen Street, Suite 1441, Newark, NJ 07107.** Priority will be given to letters of interest and curricula vitae received by February 1, 2011. If you have any questions, please contact: **Dr. Andrew Thomas, Chair, Search Committee, 973-972-1396; [thomasap@umdnj.edu](mailto:thomasap@umdnj.edu).** Equal Opportunity Employer.



**UMDNJ**  
UNIVERSITY OF MEDICINE &  
DENTISTRY OF NEW JERSEY



***Science Careers*** is the window  
that displays your vision.



Visit our  
**ENHANCED**  
WEBSITE!

Revealing your vision to employers is our job. We're your source for connecting with top employers in industry, academia, and government. We're the experts and entry point to the latest and most relevant career information across the globe.

Our newly designed website offers a set of tools that reveal career opportunities and your personal potential. Whether you're seeking a new job, career advancement in your chosen field, or ways to stay current on industry trends, *Science Careers* is your window to a limitless future.

**Improved Website Features:**

- » Relevant Job E-mail Alerts
- » Improved Resume Uploading
- » Content Specific Multimedia Section
- » Facebook Profile

**Job Search Functionality:**

- » Save and Sort Jobs
- » Track Your Activity
- » Search by Geography
- » Enhanced Job Sorting



*Your Future Awaits.*

**Science Careers**

From the journal *Science*



[ScienceCareers.org](http://ScienceCareers.org)



## POSITIONS OPEN

### CONSERVATION BIOLOGIST OR RESTORATION ECOLOGIST

The Biological Sciences Department at California State Polytechnic University (Cal Poly Pomona), invites applications for a tenure-track, **ASSISTANT PROFESSOR** position in Conservation Biology or Restoration Ecology, beginning September 2011. This position is intended to integrate with and contribute to our growing Environmental Biology Program. A Ph.D. in Biology or a related field, a demonstrated record of publication, and some teaching experience at the college level are required; postdoctoral experience is preferred. The area of expertise is open, but candidates with a strong field-based research program in conservation/restoration biology are particularly encouraged to apply. The successful candidate will be expected to teach courses in general ecology, conservation biology, and other upper division and/or graduate level courses related to his or her area of expertise. Teaching duties may also include introductory biology or a general education course. The successful candidate must be strongly committed to teaching, mentoring of undergraduate and graduate (M.S.) students, and developing an externally funded research program. Cal Poly Pomona is a comprehensive Master's level university with a diverse student body. The successful candidate will have demonstrated ability to be responsive to the educational equity goals of the university and its increasing ethnic diversity and international character. Applicants should mail: (1) curriculum vitae, (2) statement of teaching philosophy, (3) proposed plan of research, (4) reprints of up to three publications, and (5) the names and contact information of five references to: **Chair, Conservation Biologist/Restoration Ecologist Search Committee, Biological Sciences Department, California State Polytechnic University, 3801 West Temple Avenue, Pomona, CA 91768-4132**. Review of applications will begin on January 5, 2011. Official transcripts and three letters of reference will be required of all finalists. For full position description, please visit the Department website at [website: http://www.csupomona.edu/~biology](http://www.csupomona.edu/~biology).

*California State Polytechnic University, Pomona is an Equal Opportunity/Affirmative Action Employer.*

### PLANT CELL PHYSIOLOGIST TENURE-TRACK

The Department of Biology at California State University San Bernardino invites applications for a tenure-track position at the rank of **ASSISTANT PROFESSOR** in the area of cellular plant physiology. The successful applicant will develop an independent research program and is expected to excel in teaching core courses related to cell and plant biology at the undergraduate and M.S. levels. Candidates must have a record of published research and show potential for developing and sustaining an independent, externally funded research program involving both undergraduate and M.S. students. Candidates must have a Ph.D. in the biological sciences; postdoctoral experience is desirable. Application deadline is January 3, 2011, or until position is filled. Submit curriculum vitae, statement of research accomplishments and goals, statement of teaching philosophy, and three letters of reference to: **Dr. David Polcyn, Chair, Department of Biology, Attn: Plant Cellular Physiology Search, California State University, 5500 University Parkway San Bernardino, CA 92407**.

### ASSISTANT/ASSOCIATE PROFESSOR Harvard Medical School Beth Israel Deaconess Medical Center

Department of Obstetrics and Gynecology. The candidate should be a Ph.D. committed to research in one of the following areas: Reproductive Biology, Reproductive Endocrinology, or Gynecologic Oncology. Attractive opportunity for the candidate to establish a new program in an outstanding and vibrant environment. Send inquiries and curriculum vitae to: **John Yeh, M.D., Professor and Chairman, Obstetrics and Gynecology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; e-mail: tlcole@bidmc.harvard.edu**.

## POSITIONS OPEN

### FACULTY POSITION ASSISTANT OR ASSOCIATE PROFESSOR in Genetics/Genomics

San Diego State University's Department of Biology announces a tenure-track position in Genetics/Genomics at the associate or assistant professor level with the appointment beginning fall 2011. We seek a productive scientist who is addressing fundamental questions in genetics and/or genomics. We are especially interested in candidates who creatively bridge traditional boundaries between evolution and cell and molecular biology using state-of-the-art experimental approaches. A Ph.D. and postdoctoral experience in genetics, genomics, evolutionary biology, or a related field are required. Candidates at all levels should have a strong record of research accomplishments and funding, an active research program, current extramural (NSF, NIH, DOE, or comparable) funding, a strong publication record, and a commitment to undergraduate and graduate teaching.

Send curriculum vitae, separate statements of research and teaching interests, three representative publications, and arrange for three letters of reference to be sent to: **Genetics/Genomics Search Committee, Department of Biology, SDSU, San Diego, CA 92182-4614**. Review of applications will begin on 15 January and will continue until position is filled. For more information, see [website: http://www.bio.sdsu.edu](http://www.bio.sdsu.edu).

*SDSU is an Equal Opportunity Employer and does not discriminate against persons on the basis of race, religion, national origin, sexual orientation, gender, gender identity and expression, marital status, age, disability, pregnancy, medical condition, or covered veteran status.*

### ASSISTANT OR ASSOCIATE PROFESSOR in Cell Biology University of California at San Francisco

The Department of Cell and Tissue Biology, School of Dentistry, invites applications for a tenure-track faculty position at the Assistant or Associate Professor level. Candidates should hold a Ph.D. degree and are expected to establish a dynamic research program in eukaryotic cell biology, and to contribute to teaching and training. Areas of interest include but are not limited to cell biological mechanisms relevant to cancer, stem cells, or host-pathogen interactions, which are focus areas of our recently established department ([website: http://ctb.ucsf.edu/](http://ctb.ucsf.edu/)). The successful candidate will become a member of the UCSF Biomedical Sciences and the Oral and Craniofacial Sciences graduate programs.

Applications received by February 1, 2011, will receive preferential consideration, but the position will remain open until filled. A single PDF file including curriculum vitae, a brief description of research accomplishments, future research plans, and teaching interests should be submitted electronically to **e-mail: ctb\_search@ucsf.edu**. Three to five reference letters should be sent directly to the same e-mail address.

*UCSF seeks candidates whose experience, teaching, research, or community service has prepared them to contribute to our commitment to diversity and excellence. UCSF is an Equal Opportunity/Affirmative Action Employer. All qualified applicants are encouraged to apply, including minorities and women.*

### ASSISTANT PROFESSOR OF BIOLOGY Clarkson University

Clarkson University is seeking candidates for Assistant Professor of Biology. The successful candidate will develop a strong, externally funded research program that complements our strengths in biotechnology and teach undergraduates and graduate students. The position requires expertise in physiology, cellular and molecular biology, and an interest in developing collaborations with faculty in biochemistry, physics, or rehabilitation engineering. For additional information about this position and to apply please visit [website: http://www.clarkson.edu/hr](http://www.clarkson.edu/hr) and click "Career Opportunities." *Clarkson University is an Equal Opportunity/Affirmative Action Employer.*

## POSITIONS OPEN

### THREE FULL-TIME TENURE-TRACK ASSISTANT PROFESSOR POSITIONS in Discipline-Based Science Education Research

The University of Maine invites applications for three full-time tenure-track faculty positions at the Assistant Professor level with expertise in discipline-based education research. The positions will be in the fields of (1) Physics, Chemistry, or Mathematics; (2) Biology, Earth Sciences, or Marine Sciences; and (3) Science Education. These positions were created through the Maine Physical Sciences Partnership (PSP) Project, under a grant to the Maine Center for Research in STEM Education to target the teaching and learning of science in grades 6-9 as well as the preparation of science teachers at the University of Maine. By the time of appointment, the candidate must have an earned Doctorate in the discipline, in discipline-based education research, or in a closely related field; strong experience in discipline-based education research; and demonstrated excellence in teaching. The new faculty member will be expected to (1) engage in research, writing, and other scholarly activities that contribute to discipline-based education research; (2) teach undergraduate- and graduate-level courses; (3) supervise graduate and undergraduate research; (4) develop an externally-funded research program; (5) serve as a student advisor; and (6) participate in leadership for collaborative efforts in STEM-education with colleagues in the home department, the Maine RiSE Center, the University, and other professional communities. The positions are academic year appointment with preferred starting date no later than September 1, 2011. See [website: http://www.umaine.edu/center/positions/PSPfaculty](http://www.umaine.edu/center/positions/PSPfaculty) for full position announcements and application instructions. Review of applications will begin January 3, 2011, and continue until the positions are filled. Incomplete applications cannot be considered. Appropriate background checks will be required.

*The University of Maine is an Equal Employment Opportunity/Affirmative Action Employer and is committed to excellence through diversity in its faculty, staff and students. We strongly encourage all qualified individuals to apply.*

### ANIMAL PHYSIOLOGIST TENURE-TRACK

The Department of Biology at California State University San Bernardino invites applications for a tenure-track position at the rank of **ASSISTANT PROFESSOR** in the area of animal physiology. The successful applicant will develop an independent research program, and is expected to excel in teaching core courses related to organismal and human physiology at the undergraduate and M.S. levels. Candidates must have a record of published research and show potential for developing and sustaining an independent, externally funded research program involving both undergraduate and M.S. students. Candidates must have a Ph.D. in the biological sciences; postdoctoral experience is desirable. Application deadline is January 3, 2011, or until position is filled. Submit curriculum vitae, statement of research accomplishments and goals, statement of teaching philosophy, and three letters of reference to: **Dr. David Polcyn, Chair, Department of Biology, Attn: Animal Physiology Search, California State University, 5500 University Parkway San Bernardino, CA 92407**.

### PRECEPTOR IN THE LIFE SCIENCES Harvard University

Harvard University is seeking a preceptor for a large introductory undergraduate course that integrates genetics, genomics, and evolution. The preceptor is responsible for designing the laboratory and discussion components, preparing course assessments, and supervising teaching fellows. The applicant must have a Ph.D. in a Life Sciences field with proven experience teaching undergraduates. For more information, see [website: http://www.mcb.harvard.edu/Jobs/TeachingFellows.php](http://www.mcb.harvard.edu/Jobs/TeachingFellows.php).

# Research Opportunities in Luxembourg. See what's behind.



## PEARL

LUXEMBOURG'S RESEARCH PROGRAMME FOR INTERNATIONALLY RECOGNISED SENIOR RESEARCHERS

If you are an **internationally recognised senior researcher**, our **research programme PEARL** gives you the opportunity to transfer your research programme to a public-sector research institution in Luxembourg and thus to accelerate the development of and to strengthen Luxembourg's research priorities. 3-5M€ are offered to Luxembourg's public-sector research institutions through this programme to compete for the best candidates. The FNR foresees to grant 1 to 2 PEARL awards per year. The call is open all year.



## ATTRACT

LUXEMBOURG'S RESEARCH PROGRAMME FOR OUTSTANDING YOUNG RESEARCHERS FROM ALL OVER THE WORLD

If you are an **outstanding young researcher**, our **research programme ATTRACT** helps you to set up an independent research team within a public-sector research institution in Luxembourg. The innovation, dynamism and creativity of your project as well as its high scientific quality should enhance Luxembourg's position in the international world of R&D. Projects selected under ATTRACT have a lifespan of five years and the financial contribution will be up to 1.5M€. The 5<sup>th</sup> ATTRACT Call will be launched in December 2010.

More information about ATTRACT and PEARL as well as the other funding opportunities offered by the National Research Fund Luxembourg can be found on the FNR's website. Go and see what's behind on [www.fnr.lu](http://www.fnr.lu)



Fonds National de la  
Recherche Luxembourg

INVESTIGATING FUTURE CHALLENGES

## PRIZES

### FERNANDO GIL INTERNATIONAL PRIZE FOR THE PHILOSOPHY OF SCIENCE 2011



The creation of the **FERNANDO GIL INTERNATIONAL PRIZE** for the Philosophy of Science was announced by the Portuguese government at the time of his death, to honor his memory and work (1937-2006).

The prize is launched as a joint initiative of the Portuguese government, represented by FCT, the Portuguese Foundation for Science and Technology (Ministry of Science, Technology and Higher Education), and FCG, the Calouste Gulbenkian Foundation.

The Fernando Gil International Prize will be awarded every year in Lisbon. The 2010 prize was awarded to Ladislav Kvasz, a Slovak Mathematician and Philosopher.

The Prize intends to award a work of particular excellence in the domain of the Philosophy of Science, whether regarding general epistemological problems or particular scientific areas.

For the prize, it will be solely considered original and recent works of a single author from any nationality or professional affiliation, published during the three previous years (2008, 2009 and 2010).

The recipient of the Prize is requested to deliver an original public lecture that will be published by the Calouste Gulbenkian Foundation and to conduct a specialized seminar for students and researchers in Lisbon on the occasion of the Prize ceremony.

**The amount to be paid to the laureate will be 125,000 Euros.**

The jury of the Fernando Gil International Prize 2010 includes the following members:  
**Henri Atlan** Hadassah University Hospital, Jerusalem & École des Hautes Études en Sciences Sociales, Paris  
**Per Aage Brandt** Case Western Reserve University  
**Marcelo Dascal** Tel Aviv University  
**Vincent Descombes** École

des Hautes Études en Sciences Sociales, Paris  
**Donald Gillies** University College London  
**Giulio Giorello** Università degli Studi di Milano  
**Eberhard Knobloch** Institut für Philosophie, Technische Universität Berlin  
**Maria Filomena Molder** Departamento de Filosofia, Universidade Nova de Lisboa  
**Frédéric Nef** École des Hautes Études en Sciences Sociales, Paris  
**Jean Petitot** École des Hautes Études en Sciences Sociales, Paris  
**Bertrand Saint-Sernin** Institut de France  
**Manuel Silvério Marques** Centro de Filosofia, Universidade de Lisboa.

Nominations to the Fernando Gil International Prize 2011 should be sent to [premio-filosofia@fernando-gil.org.pt](mailto:premio-filosofia@fernando-gil.org.pt) until February 28, 2011 at 12h00 UTC, and include the following elements:

- The complete publication data of the nominated work;
- A critical review of the work being proposed for the prize emphasizing the elements considered to be more relevant for the awarding of the prize (2000 characters);
- A brief comment on the author biography (2000 characters).

Simultaneously, a paperback copy of the nominated publication should be sent to the Foundation for Science and Technology, for the address indicated below:

Foundation for Science and Technology  
c/o Andreia Rosa  
Av. D. Carlos I, 126,  
1249-074 Lisboa - Portugal

**Nominations from the nominees themselves cannot be accepted.**

For more information:  
[www.fernando-gil.org.pt](http://www.fernando-gil.org.pt)



## 8th IBRO WORLD CONGRESS OF NEUROSCIENCE

International Brain Research Organization

Florence - Italy, July 14 - 18, 2011

[www.ibro2011.org](http://www.ibro2011.org)

Registration and abstract  
submission are now open!



The 8th IBRO World Congress has been inserted in the calendar of events related to the Celebrations of the 100th Anniversary of the Publication of the Nobel Prize.



## POSITIONS OPEN

### FACULTY POSITION in Ecological or Evolutionary Genomics

Saint Louis University, a Catholic, Jesuit institution dedicated to student learning, research, health, and service, is seeking applicants for a tenure-track faculty position in Ecological or Evolutionary Genetics/Genomics in the Department of Biology. Competitive applicants will have a Ph.D., postdoctoral experience, a record of research productivity, and a commitment to undergraduate and graduate student training. The successful candidate will be expected to establish an independent, extramurally funded research program that applies genetic and/or genomic approaches to fundamental questions in ecology or evolutionary biology. The faculty member will contribute to an undergraduate course in Genetics, a graduate course in the candidate's area of expertise, and/or a general biology course.

All applications must be made online at **website: <http://jobs.slu.edu>** (Req ID 20100990) and include a cover letter, curriculum vitae, a research statement, and a statement of teaching experience and philosophy. In addition, please have three letters of reference sent to: **Dr. Robert Wood, Department of Biology, Saint Louis University, 3507 Laclede Avenue, St. Louis, MO, 63103**. Review of applications will begin on 15 December 2010, and continue until the position is filled. Additional information on the Department of Biology can be found at **website: <http://www.slu.edu/x14762.xml>**.

*Saint Louis University is an Affirmative Action/Equal Opportunity Employer (AA/EOE), and encourages nominations of and applications from women and minorities.*

### VISITING ASSISTANT PROFESSOR OF BIOLOGY

Kenyon College is seeking applications for an appointment as a two-year Visiting Assistant Professor of Biology. The teaching commitment is five courses per year; laboratories count as separate courses. The successful candidate will be expected to teach introductory and advanced courses in animal physiology or ecology, including lecture and laboratory courses. There are opportunities for undergraduate research mentoring and support for professional development.

To apply, candidates should visit the online application site found at **website: <https://employment.kenyon.edu>**. A complete application will comprise: (1) a cover letter discussing the applicant's research and scholarship undertaken, its relevance to the field or discipline, and prior teaching experience; (2) a statement of the applicant's teaching philosophy; (3) curriculum vitae; (4) unofficial graduate and undergraduate transcripts; and (5) letters of reference from three (3) recommenders. All application materials must be submitted electronically through Kenyon's employment website.

Review of applications will begin January 15, 2011, and will continue until the position is filled. Completed applications received by the January 15 deadline will be guaranteed full consideration. A Ph.D. in Biology or related field is required.

### ASSISTANT PROFESSOR, ANATOMY

The Biological Sciences Department at the University of Wisconsin-Parkside invites applications for a Tenure-Track Assistant Professor (9 month) position in Anatomy, beginning August 2011. Primary responsibilities include: teaching, research, and service. Qualifications include: Ph.D., research experience, teaching experience, and sensitivity to, or experience in, working with a diverse, multicultural population. Applications received by February 1, 2011, are ensured full consideration; position is open until filled. For more details visit **website: <http://www.uwp.edu/departments/human.resources/unclassified.positions/index.cfm>**.

*UW-Parkside is an Affirmative Action/Equal Employment Opportunity Employer D/M/V/W.*

## POSITIONS OPEN

### TENURE-TRACK ASSISTANT PROFESSOR in Human Physiology

The Department of Biology, University of Wisconsin-Stevens Point, offers a tenure-track, nine-month faculty position in Human Physiology (Assistant Professor), beginning August 2011. Teaching assignment includes human physiology, introductory biology, senior seminar, and, eventually, an advanced course in specialty. Research involving undergraduates, department service, and student advising are expected. Ph.D. with emphasis in mammalian physiology (for tenure), and teaching and research experience are required. Coursework and/or research in neurobiology is desirable. Experience may include grants, publications, evidence of teaching excellence, and postdoctoral work. The faculty seeks applicants from underrepresented groups.

Include curriculum vitae, statements of teaching philosophy and research interests, three letters of recommendation, and undergraduate and graduate transcripts. Send application materials to: **Dr. C. Yahnke, Chair, Biology Department, University of Wisconsin-Stevens Point, Stevens Point, WI 54481**. Review begins 1 February 2011, and continues until filled. For more information, **telephone: 715-346-2455; fax: 715-346-3624; e-mail: [cyahnke@uwsp.edu](mailto:cyahnke@uwsp.edu)**.

*Affirmative Action/Equal Opportunity Employer.*

### POSTDOCTORAL POSITIONS in Cancer Biology and Therapeutics

Several postdoctoral positions are available in the area of antibody engineering and cancer, including the use of advanced fluorescence microscopy and murine cancer models to develop improved therapeutics. Strong applicants with a Ph.D. in biological or physical sciences, including bioengineering, will be considered. Please send curriculum vitae as well as names and contact information of three references to: **Professor E. Sally Ward (e-mail: [wardlab@utsouthwestern.edu](mailto:wardlab@utsouthwestern.edu))**. *UT Southwestern is an Equal Opportunity/Affirmative Action Employer.*

Do what  
you love.

Love what  
you do.

[www.sciencecareers.org](http://www.sciencecareers.org)

Science Careers

From the journal *Science*



A Career  
in science  
is more  
than just  
science.

[www.sciencecareers.org](http://www.sciencecareers.org)

Science Careers

From the journal *Science*



Find your  
future here.



Science Careers

From the journal *Science*



[www.ScienceCareers.org](http://www.ScienceCareers.org)

## MARKETPLACE

Widely  
Recognized  
Original &  
Guaranteed

**KlenTaq1**

8¢/u  
Truncated  
Taq DNA  
Polymerase  
Withstand 99°C

US Pat #5,436,149  
Call: **Ab Peptides**  
Fax: 314•968•8988

e-mail: [abpeps@msn.com](mailto:abpeps@msn.com)  
1•800•383•3362  
[www.abpeps.com](http://www.abpeps.com)

Promab Biotechnologies Inc.

**Custom Monoclonal  
Antibody \$4,200**

>3,000 CLONES WILL BE SCREENED

1-866-339-0871

[www.promab.com](http://www.promab.com) [info@promab.com](mailto:info@promab.com)

ambion® | RNA  
by *life* technologies™

*life*

**purity**  
it's in our RNA



### Ambion... superior results every time.

Ambion is the RNA expert. For over 20 years, our scientists have developed high-quality, innovative RNA products that make your job easier with faster, more robust and reliable results. Use the best and have confidence in your results...every time. Ambion, including Invitrogen™ RNA products, is now part of Life Technologies.



go to [www.invitrogen.com/ambionRNA](http://www.invitrogen.com/ambionRNA)

download the free mobile app at <http://gettag.mobi>  
scan the barcode to instantly access more information about RNA

*life*  
technologies™

Life Technologies offers a breadth of products DNA | RNA | protein | cell culture | instruments

FOR RESEARCH USE ONLY. NOT INTENDED FOR ANY ANIMAL OR HUMAN THERAPEUTIC OR DIAGNOSTIC USE, UNLESS OTHERWISE STATED.

© 2010 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners, unless otherwise noted.





"How do we know  
this lead molecule  
is novel?"

“SciFinder—  
of course.”

#### Need to assess the novelty of substances?

SciFinder is the answer.

It includes CAS REGISTRY<sup>SM</sup> the most comprehensive substance information available, integrated with relevant journal articles and patents.

Give your research team the highest quality and most timely scientific information resource.

Make SciFinder an essential part of your research process.

For more information about SciFinder, visit [www.cas.org](http://www.cas.org) or e-mail [help@cas.org](mailto:help@cas.org).

*an essential*  
✓  
**SciFinder®—Part of the process.™**



SciFinder®

[www.cas.org](http://www.cas.org)

GE Healthcare  
Life Sciences

# Inspired to Accelerate scale-up

Inspired by your need for fast and predictable scale-up, we introduce ÄKTA™ avant 150, the latest development in our ÄKTA avant series.

Optimize your process with ÄKTA avant 25 and then scale-up seamlessly to ÄKTA avant 150 by automatically converting your methods using UNICORN™ 6.1 software.

ÄKTA avant is designed to fully exploit the advantages of our modern BioProcess™ media like MabSelect™ and Capto™.

Dedicated column formats ensure seamless scalability from process development to manufacturing – from prepacked HiScreen™ columns to HiScale™ to AxiChrom™ with Intelligent Packing.

Want to know more? Register today to receive a copy of the ÄKTA avant 150 Data File.  
[www.gelifesciences.com/pr-avant](http://www.gelifesciences.com/pr-avant)



imagination at work

GE, Imagination at work, and GE monogram are trademarks of General Electric Company.  
ÄKTA, AxiChrom, BioProcess, Capto, HiScale, HiScreen, MabSelect, and UNICORN are  
trademarks of GE Healthcare companies.  
© 2010 General Electric Company – All rights reserved.  
First published Sept. 2010  
GE Healthcare Bio-Sciences AB, Björksgatan 30, 751 84 Uppsala, Sweden  
GE09-10





# Any sample, any application — no limits

**Maximize success  
with QIAGEN sample technologies**

- Innovative, room-temperature sample collection and stabilization
- DNA, RNA, and protein purification from any sample
- Hands-free automated sample preparation
- Reliable genetic, epigenetic, and gene expression analysis from FFPE samples
- Whole genome and transcriptome amplification to overcome sample limitations

Contact QIAGEN today or visit [www.qiagen.com/sample-technologies](http://www.qiagen.com/sample-technologies)



**Sample & Assay Technologies**



**Pioneering** Humanity is still forging ahead by reflecting on its position from revolutionary perspectives. Leica Microsystems has mapped its corporate values. For more information, visit our website.

## Professor Hell, what awaits us beyond the diffraction limit?

Stefan Hell dared to think the unthinkable: nanometer resolution in the focal plane of a light microscope. Today this has become routine. Now it's time to do the unthinkable again! For the discovery of new worlds in the nanostructures of life.

[www.leica-microsystems.com](http://www.leica-microsystems.com)

## Living up to Life

**Leica**

MICROSYSTEMS

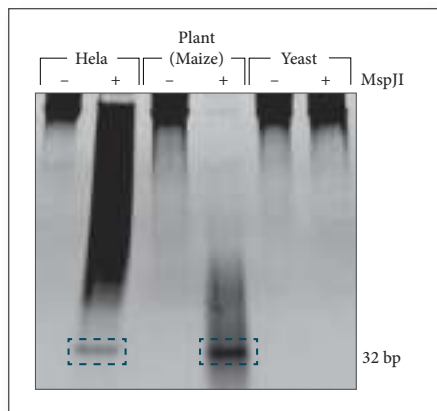


## UNDERSTANDING CHANGE

### New tools to advance epigenetics research

For over 35 years, New England Biolabs has been committed to understanding the mechanisms of restriction and methylation of DNA. This expertise in enzymology has recently led to the development of a suite of validated products for epigenetics research. These unique solutions to study DNA methylation are designed to address some of the challenges of the current methods. EpiMark™ validated reagents simplify epigenetics research and expand the potential for biomarker discovery.

Simplify DNA methylation analysis with MspJI



*MspJI recognizes methylated and hydroxymethylated DNA and cleaves out 32 bp fragments for sequencing analysis. Overnight digestion of 1 µg of genomic DNA from various sources with or without MspJI is shown. Note: Yeast DNA does not contain methylated DNA, therefore no 32-mer is detected.*

**EpiMark™ validated products include:**

- Newly discovered methylation-dependent restriction enzymes
- A novel kit for 5-hmC and 5-mC analysis and quantitation
- Methyltransferases
- Histones
- Genomic DNAs

To learn how these products can help you to better understand epigenetic changes, visit [neb.com/epigenetics](http://neb.com/epigenetics).



CLONING & MAPPING

DNA AMPLIFICATION  
& PCR

RNA ANALYSIS

PROTEIN EXPRESSION  
& ANALYSIS

GENE EXPRESSION  
& CELLULAR ANALYSIS

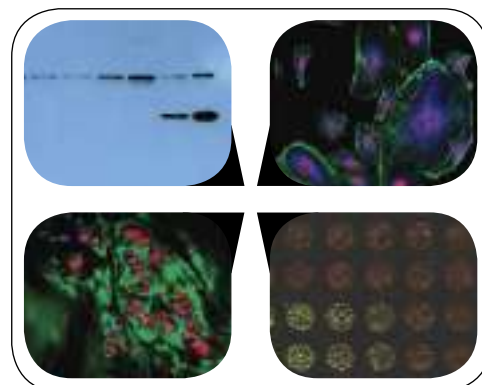
[www.neb.com](http://www.neb.com)



## Share your data and get a free antibody!

Introducing the Thermo Scientific Pierce Antibody Innovators Program where you are rewarded for sharing your data with us and others. The no-hassle program provides several options to submit your data and, in return, you could receive additional antibodies of equal or lesser value absolutely free. As always, you can rely on our 100% performance guarantee that ensures all antibodies work robustly in the applications listed on the data sheet.

Visit [www.thermoscientific.com/innovatorsprogram](http://www.thermoscientific.com/innovatorsprogram) to learn how to submit your data for this great rewards program and to view our extensive antibody portfolio.



**Free antibodies!** Share your data with us and you could get your next antibody free. Choose from more than 30,000 antibodies in 42 research areas.



# The largest catalog of the best antibodies in the world

Abcam provides more than 66,000 cutting-edge antibodies with the most comprehensive and up-to-date datasheets including customer reviews and antibody-specific protocols.

- Primary antibodies • Lysates • Secondary antibodies
- Proteins/Peptides • Kits • Slides

[www.abcam.com](http://www.abcam.com)



- **Embryonic and mesenchymal stem cell panels**  
Designed for the validation and characterization of cultured or newly derived embryonic stem (ES) cell lines and cultured or isolated multipotent mesenchymal stromal cells.

[www.abcam.com/stemcell\\_panel](http://www.abcam.com/stemcell_panel)

- **New fluorochrome range of conjugates**

Chromo™, DyLight® and SureLight® fluorochromes. These fluorescent dyes exhibit higher photostability and lower background staining.

[www.abcam.com/secondary\\_antibody](http://www.abcam.com/secondary_antibody)

- **EXPOSE – biotin-free IHC detection systems**

Greater signal and higher sensitivity: allows greater antibody access to targets compared to conventional polymer amplified IHC.

[www.abcam.com/IHC\\_kitsplusreagents](http://www.abcam.com/IHC_kitsplusreagents)

## Downloadable pathways and posters

We work hard to gather new pathways that complement our large range of antibodies, which you can download or request hard copies by mail. Check them out today!

[www.abcam.com/poster](http://www.abcam.com/poster)

DyLight® is a trademark of Thermo-Fisher Scientific Inc. and its subsidiaries.

## Welcome to Abcam

The largest catalog of the best antibodies

Product search:

Products by research area:

|                       |                      |
|-----------------------|----------------------|
| Cancer                | Microbiology         |
| Cellular Biology      | Neuroscience         |
| Cell Biology          | Reproductive Biology |
| Chemistry             | Signal Transduction  |
| Developmental Biology | Stem Cells           |
| Immunology            |                      |



Learn more at

[www.abcam.com/abpromise](http://www.abcam.com/abpromise)

## Contact Abcam:

Phone: 1-888-77-ABCAM

E-mail: [technical@abcam.com](mailto:technical@abcam.com) | [us.orders@abcam.com](mailto:us.orders@abcam.com)

## General office hours:

Customer service: 8:30am - 5pm EST Mon - Fri

Scientific support: 8:30am - 5pm EST Mon - Fri

# Imagine if Otto Warburg had a Seahorse XF Extracellular Flux Analyzer...



TheScientist 2009  
**TOP 10**  
INNOVATIONS

Finally, a real-time, kinetic measurement of the **Warburg Effect**, glucose & glutamine addictions, and fatty acid oxidation of cancer cells in a microplate.

## Seahorse's award winning XF Extracellular Flux Analyzers provide an easy way to:

- Evaluate the role of oncogenes and tumor suppressor genes in energy metabolism and tumorigenesis
- Detect HIF-1 and drug mediated effects on mitochondrial respiration and glycolysis
- Determine which energy substrates are preferentially used by tumor cells
- Measure the dynamic contributions of OXPHOS & aerobic glycolysis
- Validate genes that target tumor metabolism

### Webinars On-Demand

See our recent cancer related webinars

#### ***Mitochondria Regulate Cancer***

Navdeep S. Chandel, PhD  
Northwestern Medical School

#### ***Metabolic Reprogramming Therapy in the Treatment of Cancer***

Nicholas Denko, MD, PhD  
Stanford University, School of Medicine  
at [www.seahorsebio.com/webinars](http://www.seahorsebio.com/webinars)

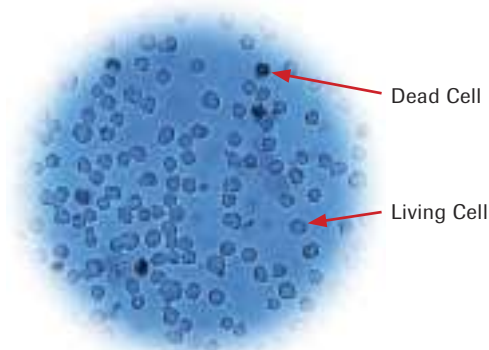
Measuring **cancer metabolism** is so easy now!



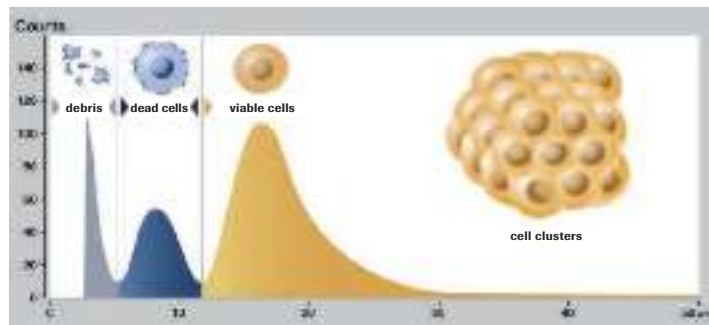


**Cedex XS Analyzer and CASY Systems**  
**NEW! from Roche**

# Count and Analyze Your Cells for Consistent Results



**Cedex Trypan Blue Exclusion Method.** Image-based analysis of cell viability using industry-proven Cedex 2 software.



**CASY High-Resolution Size Distribution Analysis.** Pulse area analysis with Electrical Current Exclusion (ECE) distinguishing cell debris, dead cells, viable cells, and aggregates.

**For life science research only.**  
**Not for use in diagnostic procedures.**

CEDEX, CASY, and INNOVATIS are trademarks of Roche.  
 Other brands or product names are trademarks of their respective holders.

© 2010 Roche Diagnostics. All rights reserved.

Improve cell culture success and consistency of experimental results by better understanding the status of your cells. With Roche Innovatis **automated multiparameter quality control**, you can monitor your cells and optimize growth conditions through fast, accurate cellular analysis.

- **Obtain accurate results** in 20 seconds with only 10 µl of cell sample!
- Use innovative **Cedex XS Analyzer** Smart Slide technology to rapidly measure cellular parameters with trypan blue exclusion and high-resolution digital imaging.
- Choose a **CASY System** to measure cellular parameters using label-free, non-invasive Electrical Current Exclusion (ECE) and high-resolution size distribution analysis.

More precisely define the state of your cells *in vitro* by using the **Cedex XS Analyzer** or **CASY Systems** – now available from Roche.

For detailed product information and application notes, visit  
[www.roche-applied-science.com](http://www.roche-applied-science.com)

Roche Diagnostics Corporation  
 Roche Applied Science  
 Indianapolis, Indiana





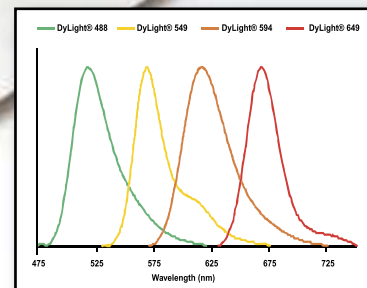
# What color will help you today?

Abcam offers hundreds of DyLight® conjugated secondary antibodies that complement our products in a wide range of applications.

- Bright fluorescence
- Excellent photostability
- Stable at pH4 - 9
- Pre-adsorbed formats

For all your secondary antibody needs visit:  
[www.abcam.com/secondary\\_antibody](http://www.abcam.com/secondary_antibody)

DyLight® is a trademark of Thermo Fisher Scientific Inc. and its subsidiaries.



**Emission spectra of DyLight® fluorochromes.**

Line colors represent the approximate visible colors of the wavelength maxima.

## Abcam Inc.

1 Kendall Square, Ste 341  
 Cambridge, MA 02139-1517  
 USA

Tel: 1-617-225-2272  
 Toll free: 1-888-77-ABCAM  
 Toll free Fax: 1-866-739-9884





## RELAX. YOU'VE GOT ENDNOTE X4.

Take a deep breath and **RELAX** while Thomson Reuters EndNote is hard at work connecting you to high quality resources, simplifying your collaboration with colleagues and removing the reference stress from all your research projects.



For efficiently gathering references and full text, no other solution delivers the rich support found in EndNote X4. Simply point EndNote toward your PDF files and folders to create new references without typing or searching. Or, let EndNote save you time locating and attaching full text PDF files for your existing references. Either way you'll have more time on your hands.

With the Web you can share EndNote reference groups and even manage your personal publication list for the free ResearcherID author community. Imagine a simple way to present your work publicly along with citation metrics delivered by the Web of Knowledge.<sup>SM</sup>

Learn about more new features in EndNote X4. You just can't **RELAX** without it.

800-722-1227 • 760-438-5526 • [rs.info@thomson.com](mailto:rs.info@thomson.com)

Download your free demo  
or buy online today  
[www.endnote.com](http://www.endnote.com)



THOMSON REUTERS

© Copyright 2010 Thomson Reuters.  
EndNote is a registered trademark of  
Thomson Reuters. All trademarks are  
the property of their respective companies.



## AAAS is here – helping educators make informed decisions.

For many K-12 teachers and librarians, tight budgets mean every purchase needs to deliver maximum value in the classroom. With *Science Books & Films (SB&F)* AAAS provides educators a way to find the best resources and the best values. With hundreds of book and movie reviews *SB&F* helps science educators bring the best tools to their schools. As a AAAS member your dues support these efforts.

If you're not yet a AAAS member, join us. Together we can make a difference.

To learn more,  
visit [aaas.org/plusyou/sbf](http://aaas.org/plusyou/sbf)



# Bio**perfection.**

Sigma's Prestige Antibodies® could prove  
priceless to your research.



Sigma now offers over 10,000 Prestige Antibodies  
powered by Atlas Antibodies.

1,700 new Prestige Antibodies are now available! Developed through the Human Protein Atlas Project, Prestige Antibodies are standardized in universal protocols to enhance the efficiency and effectiveness of your research. Each antibody is supported with over 700 immunohistochemistry, immunofluorescence and Western blot images that are all publicly available on the Human Protein Atlas website.

[wherebiobegins.com/prestige](http://wherebiobegins.com/prestige)

**Prestige Antibodies®**

Powered by  **ATLAS**  
ANTIBODIES

**SIGMA-ALDRICH®**

**SIGMA®** Where *bio* begins™  
Life Science

Prestige Antibodies powered by Atlas Antibodies is a registered trademark of Sigma-Aldrich and Sigma-Aldrich Biotechnology, LP.





# Next-gen GWAS. NOW.

It's a content revolution.

Up to 50% more coverage of common and rare variants than all other arrays. Maximum power for any population.

The Omni family of microarrays can propel your studies into true next-gen GWAS. With a clearly defined product path to the future. Immediate utility. Future flexibility.

Get on the path to next-gen GWAS.

Now is the time. Get started at

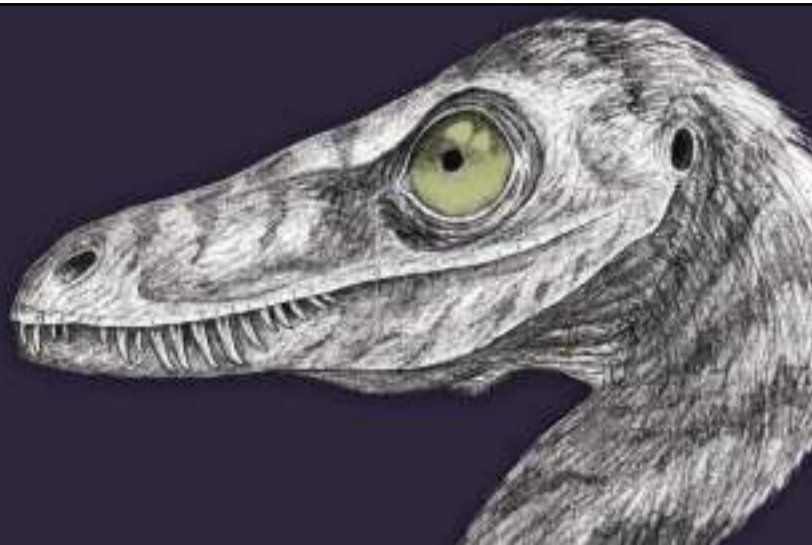
[www.illumina.com/GWAS](http://www.illumina.com/GWAS)

# Call for 2010 Cozzarelli Prize Nominations



The PNAS Editorial Board is now accepting nominations through January 14, 2011, for the 2010 Cozzarelli Prize. This award recognizes scientific excellence and is given to six papers published in PNAS during the past year.

Nominations should include a citation and brief explanation of the merits of the work. The award recipients will be recognized during the PNAS Editorial Board Meeting and the NAS Annual Meeting Awards Ceremony on May 1, 2011, in Washington, DC.



*For more information and a list of  
previous winners visit:  
[www.pnas.org/site/misc/cozzarelliprize.shtml](http://www.pnas.org/site/misc/cozzarelliprize.shtml)*

**PNAS**  
[www.pnas.org](http://www.pnas.org)



# innovating



## AstraZeneca excellence in chemistry award 2010

AstraZeneca Pharmaceuticals is proud to present the 2010 Excellence in Chemistry Awards. We recognize that advances in medicine rely on innovations in chemistry. For twenty-six years, we have demonstrated our commitment to innovation by awarding two talented academic researchers affiliated with universities in the United States and Canada who, early in their careers, have made outstanding contributions to synthetic, mechanistic, or bio-organic chemistry.

[astrazeneca.com](http://astrazeneca.com)

Pictured left to right:

**Professor Christopher Vanderwal**  
University of California, Irvine

Distinguished Lecturer:  
**Peter R. Bernstein, Ph.D.**  
AstraZeneca, Retired

**Professor Tobias Ritter**  
Harvard University





## 3<sup>rd</sup> International Conference on Drug Discovery & Therapy

February 7 - 10, 2011, Dubai, U.A.E.

# Be a Part of the Drug Discovery Venture!

---

### PARTICIPATING NOBEL LAUREATES







Michael S. Brown   Robert H. Roberts   Ewen F. Fyfe   Harold M. Holt   J.M. Loh

### SELECTED TRACKS

- Antimicrobials
- Anti-Cancer Discovery & Therapy
- Biologics
- Cardiovascular Drug Discovery & Therapy
- CNS Drug Discovery & Therapy
- Drug Delivery & Targeting
- Drug Discovery in Preclinical Research
- Drug Metabolism
- Hot topics in Drug Targets
- Hot topics in Medicinal Chemistry
- Translational Medicine

### CONFERENCE PRESIDENTS




Prof. Fred Meret  
(Nobel Laureate)

Prof. Dr. Atip-un-Kashman  
FNS

#### KEY BENEFITS OF ATTENDING

- Explore Cutting-Edge Discoveries
- Gain Networking Opportunities
- Focus on Pre-Clinical & Therapeutic Themes
- Commercial Exhibition

#### TO KNOW MORE ABOUT

- Registration
- Submission of Abstracts
- Sponsorship & Exhibition Opportunities

please visit the event website

www.icddt.com

Contact: ICDDT 2010, Secretariat  
PO Box 121223, SAIF Zone, Sheikh U.A.E.  
Tel: +971-6-3573763, Fax: +971-6-5575764  
Email: [marketing@icddt.com](mailto:marketing@icddt.com)

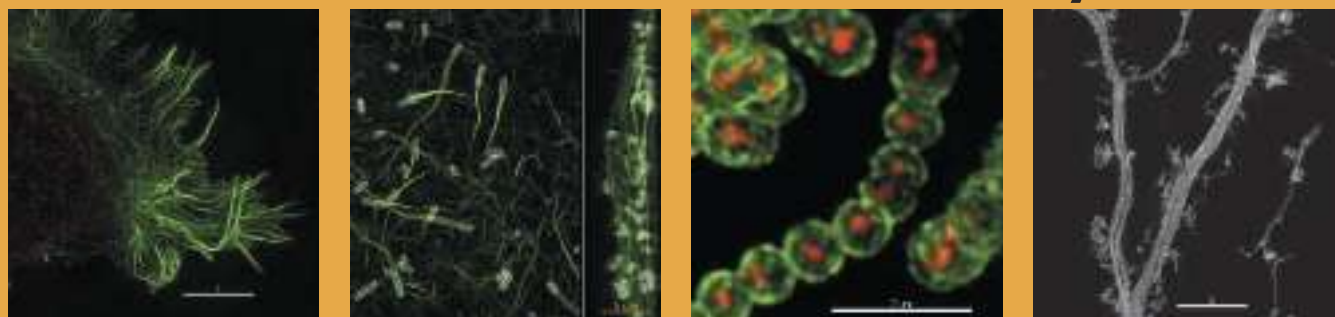
NATIONAL ORGANIZER — INTERNATIONAL ORGANIZER




Real data.  
Real installations.  
Real super-resolution imaging.



# Really.



Learn more about the DeltaVision OMX® super-resolution imaging system at [www.super-resolution.com](http://www.super-resolution.com).

CHAPMAN UNIVERSITY CONGRATULATES

# DR. YAKIR AHARONOV

2010 NATIONAL MEDAL OF SCIENCE RECIPIENT



Awarded by the president of the United States, the National Medal of Science is the nation's highest scientific honor. It is given to "individuals deserving of special recognition by reason of their outstanding contributions to knowledge in the physical, biological, mathematical, or engineering sciences."



Dr. Aharonov holds the James J. Farley Professorship in Natural Philosophy in Chapman University's Schmid College of Science. Together with the late David Bohm, Ph.D., Dr. Aharonov co-discovered the Aharonov-Bohm Effect, which is regarded as one of the cornerstones of modern physics.

The presidential citation accompanying Dr. Aharonov's medal recognizes him for "his work in quantum physics which ranges from the Aharonov-Bohm effect, to the notion of weak measurement, making him one of the most influential figures in modern physics."

Chapman University continues to advance critical research and education in the sciences, building expertise in health and life sciences, computational sciences, and earth and environmental sciences.

Dr. Aharonov's presence at Chapman University continues to enrich the intellectual life of our campus and our students. We join his peers and the President of the United States in saluting Dr. Aharonov for this extraordinary accomplishment.



Orange, California | [chapman.edu](http://chapman.edu)



2nd  
registration  
half price

# Therapeutic approaches to neurodegeneration- age modifiers, proteostasis and stem cells

## Venue & Date:

Sheraton Nassau Beach Resort  
February 14-17, 2011

## Topics:

Aging, proteostasis and stem cells

## Invited Speakers:

Arturo Alvarez-Buylla | Grigori Eriokopov | Anne Brunet  
Sangram Sisodia | Marc Diamond | Mathias Jucker  
Paul Taylor | Rudy Tanzi | Rick Morimoto | Andy Dillin  
Sean Morrison | Jens Brünig | Juan Carlos Belmonte  
Leonard Guarente | David Holtzman | Peter Reinhart  
Paul Muchowski | Fiona Doetsch

## Organized by:

Andy Dillin, David Holtzman, Sean Morrison and Abcam



## Special Promotion

Register one person,  
get the second  
person half price!

[www.abcam.com/neuro2011](http://www.abcam.com/neuro2011)

2nd  
registration  
half price

## Terms and conditions:

Register through the website at:  
[www.abcam.com/neuro2011](http://www.abcam.com/neuro2011)  
Please Email: [events@abcam.com](mailto:events@abcam.com)  
to receive the 2nd person half price.  
Confirmation emails will be sent out.  
Terms and conditions apply.  
Valid for full registrations received by  
**December 31, 2010 only**. In the event  
of cancellation, standard conference  
terms and conditions will apply and you  
will be liable for the full cost of the 2nd  
registration. This Cannot be used in  
conjunction with any other offer. Abcam  
reserves the right to remove this offer at  
any time without prior notice.





# TruSeq<sup>TM</sup>

The most accurate  
next-gen sequencing  
technology available.

Every Illumina sequencer is powered by TruSeq—the technology that delivers the most accurate human genome at any coverage. TruSeq produces the highest yield of error-free reads. The most bases over Q30. The greatest number of peer-reviewed publications—more than 1,000 in the past three years.

That's Tru data quality.

Get the proof. Go to

[www.illumina.com/TruSeq](http://www.illumina.com/TruSeq)



HiSeq 2000



HiSeq 1000



HiScan SQ



Genome Analyzer<sub>IIx</sub>

illumina<sup>®</sup>

CREATING NEW KNOWLEDGE THROUGH

Images courtesy UW-Madison



WISCONSIN INSTITUTES FOR  
**DISCOVERY**

MORGRIDGE INSTITUTE FOR RESEARCH

WISCONSIN INSTITUTE FOR DISCOVERY

TOWN CENTER

Now open in the heart of the  
University of Wisconsin–Madison

[www.discovery.wisc.edu](http://www.discovery.wisc.edu)

COLLABORATION

LIFE SCIENCE TECHNOLOGIES

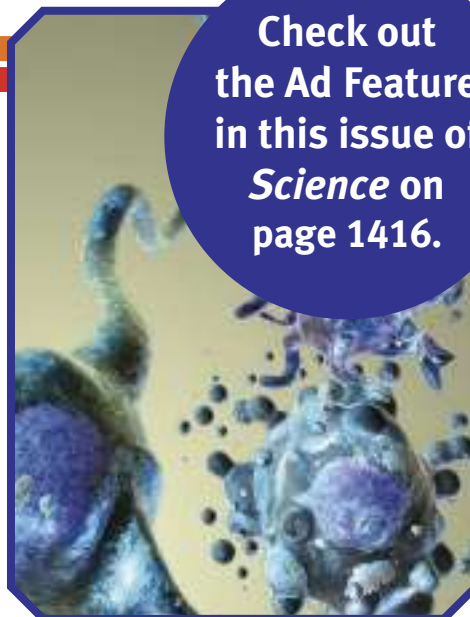
## Understanding Apoptosis

This feature explores the technologies that are available for studying apoptosis, or programmed cell death, from the basic assays that offer simple, single molecular event detection to more recent techniques offering high throughput, multiplexing capabilities, and in vivo imaging. These technological advances not only help scientists understand this complex cellular process, but also have the potential to aid in tailoring and monitoring personalized treatments for a variety of diseases, especially cancer.

May also be seen online at [www.sciencemag.org/products/articles.dtl](http://www.sciencemag.org/products/articles.dtl).

Produced by the *Science*/AAAS Business Office

Check out  
the Ad Feature  
in this issue of  
*Science* on  
page 1416.





## APOPTOSIS

# UNDERSTANDING APOPTOSIS

Apoptosis is a simple concept with complex underpinnings—damaged cells are prompted to self-destruct through a series of molecular events, like dominos toppling over one by one. Unraveling the mechanisms underlying apoptosis is akin to determining the role each individual domino plays in a falling cascade—one strategically placed tile can trigger a cascade of several lines, and one misplaced tile can mean a dead end in the chain reaction. Techniques that pick apart this intricate process have been rapidly advancing, now offering more than the ability to look at a single event with high throughput, multiplexing capabilities, and in vivo imaging. Future technologies aim to help scientists visualize human cellular processes in vivo to aid researchers in tailoring and monitoring treatment for a variety of diseases, especially cancer.

By Paul Smaglik

"Mechanism is where it's at these days."



**A**poptosis differs from necrosis, although the end results are the same—cell death. Whereas apoptosis mirrors the actions implied by the word's Greek origins for "dropping off," akin to flowers losing their petals one by one, necrosis causes a cell to abruptly explode and spill its potentially toxic contents into the surrounding environment. Scientists became fascinated with the orderly apoptotic process, or programmed cell death, because of its key role in a host of diseases—most notably in cancer, which results from rogue cells proliferating instead of self-destructing when apoptotic signals go awry.

The "dropping off" process often begins with an environmental stress, like heat, radiation, or viral infection. These stressors can initiate a molecular signaling cascade, either by moving proteins around from inside the cell to the outside or by activating proteins inside the cell, such as caspases that tell the cell to start dismantling itself. The final stages of this systematic self-destruction leave signs of the wreckage behind, for instance DNA fragments that resemble broken ladders. Each of these steps can be isolated for detection through a variety of methods.

Researchers started identifying the numerous apoptotic markers and mechanisms in the early '90's. The first kits developed to aid their search simply detected individual steps within the entire process—whether an individual domino tipped. However, subsequent generations of technology have become more sophisticated with advances that detect apoptotic markers at different stages of the cascade and identify the underlying mechanisms at play.

Scientists usually have one of two goals when studying apoptosis, says Terri Borree, **EMD Millipore's** product manager. Some seek general knowledge about apoptosis—whether it's happening, when it's triggered, and what is either causing or inhibiting it. Others want to reveal more about the process itself—perhaps by finding missing links in the domino chain of chemical events or differen-

tiating apoptosis from necrosis. However, some scientists bridge the two approaches when studying a specific disease, especially if they are investigating whether a particular compound can inhibit or promote apoptosis, says Mike Earley, market segment manager at **Sigma-Aldrich**.

## ONE AT A TIME

Although the most basic approaches for detecting apoptosis have been around for a while, "the technology hasn't changed that much," Earley explains. However, these simple assays and tools play a valuable role for researchers who simply need to confirm whether apoptosis is happening. Several stages in the apoptotic cascade have distinct events that can be easily detected, such as protein rearrangements, caspase activity, and DNA alterations.

For scientists who want a simple way to determine whether apoptosis happened, without looking at individual components of the complex pathway, Annexin V-specific stains can detect the calcium-dependent protein, which moves from the inner surface of the cytoplasm to outside the cell during apoptotic activity. This approach's speed and ease-of-use has made it one of the most popular simple assays. "The Annexin V assay is a one-step procedure requiring just 10 minutes to perform," says Suvarna Gandlur, product manager of **Clontech** in Mountain View, Colorado. "The assay is nonenzymat-

## UPCOMING FEATURES

Proteomics Meets Glycomics—January 7

Forensic Technologies—March 4

Genomics: Synthetic Genomics—March 25

ic, does not require fixation, and is suitable for both adherent and suspension cells.” Besides Clontech, many other companies offer Annexin V assays, including **Life Technologies**, **Cambrex**, and **BioVision**.

However, Annexin V assays do not tease apart the individual stages of the signaling cascade; they only detect whether or not it is underway. Caspase assays, on the other hand, can reveal more about the early stages of the process. For instance, caspase 8 and caspase 9 are involved in the induction of apoptosis, and caspase 3 plays a key role in the execution of the process. **Promega** in Madison, Wisconsin, focused its R&D efforts on developing more sensitive and easily automated bioluminescent probes to detect activation of several of these key caspases, explains Pam Guthmiller, strategic marketing manager of cellular analysis. “Mechanism is where it’s at these days,” says Guthmiller.

Scientists seeking markers of the later stages of the apoptotic cascade can look for signs of DNA fragmentation. This programmed destruction of the genome can be detected using a terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay. The technique allows researchers to look for breaks in the DNA strands, which can result from apoptosis-induced nuclease activity, by labeling the exposed 3’ hydroxyl ends. However, these assays were originally difficult to perform and could not differentiate between necrotic and apoptotic induced DNA damage.

Many academic researchers wanted clearer evidence of this end-stage, which shows that the apoptotic process reached its conclusion, says Sallie Cassel, director of product management, at EMD Millipore. In response, companies have refined these assays. For example, EMD Millipore’s ApopTag line is able to differentiate the characteristic blunt ends of DNA found only in apoptosis from the DNA overhangs created during both apoptosis and necrosis.

## MULTITASKING, MULTIPLEXING

Though assays have become more sensitive and accurate over the years, researchers also require enhanced speed, higher throughput, and increased multiplexing capabilities. As a result, companies have been adapting their technologies to multiple approaches, from antibody-based assays to flow cytometry, and expanding the number of samples that can be read at once using microwell plate readers.

The ability to detect multiple apoptotic markers simultaneously allows researchers to gain a better overview of the process, since apoptosis encompasses so many events. Promega, for example, has optimized methods that use both fluorescent and luminescent probes in one sample well. Last year, they released the ApoTox-Glo Triplex Assay, which measures viability, membrane integrity as a marker of necrosis, and caspase-3/7 activity as a marker of apoptosis, all in the same sample well. “The assay not only allows you to obtain more biologically relevant information from the same cell sample well without manipulation, but also minimizes cell culture expenses by streamlining three assays into one plate,” says Guthmiller.

Using flow cytometry, researchers can obtain multiplexed answers on a large number of samples simultaneously, explains Ja-



Scientists became fascinated with the orderly apoptotic process, or programmed cell death, because of its key role in a host of diseases—most notably in cancer, which results from rogue cells proliferating instead of self-destructing when apoptotic signals go awry.

son Whalley, flow cytometry manager at EMD Millipore. Flow cytometry also provides increased speed with the ability to “read” for multiple apoptotic markers in single cells, rather than in populations of cells, and provides a time-lapse view of what’s happening in these individual cells. This technique enables researchers to see when multiple events occur, says Terri Borree, an EMD Millipore product manager. Given the multiplicity of samples that need to be screened and the variety of treatment conditions that need to be evaluated in apoptosis studies, benchtop flow cytometers can ease researchers’ workloads.

EMD Millipore’s latest addition has been flow cytometry assays that detect mitochondrial dysfunction, which is often an early indicator of cellular stress and can trigger apoptosis or other cell death pathways. EMD Millipore’s MitoDamage Kit provides simultaneous information on mitochondrial perturbations, apoptosis, and cell death, while the MitoStress kit allows researchers to study the relationship between mitochondrial oxidative stress and apoptosis.

Life Technologies has also worked to make flow cytometry easier to use, and more effective for apoptosis research. “Given that no single parameter fully defines apoptosis in all systems, we have focused on developing and supplying products that focus on a multiparametric approach that enable researchers to not only determine the relative stage of apoptosis but to also distinguish between live, apoptotic, and necrotic cells in their experimental system,” says Kathleen Free, senior manager of product development at Life Technologies (formerly Invitrogen). These products include kits designed to go along with detection systems that have the most recent advances, such as flow cytometers with a 405 nm laser—which enhances these machines’ multiplexing capabilities—as well as high-content imaging platforms.

Life Technologies also has a novel way to label DNA, RNA, and proteins that further enhances the sensitivity of these detection technologies. The company provides small molecule labeling and detection reagents that are significantly smaller than conventional antibodies, biotin, or fluorescent tags. The Click-iT TUNEL assay, for example, uses an alkyne-modified dUTP to label fragmented DNA ends. This molecule is more easily incorporated into DNA strand breaks than the more traditional nucleotide modifications, such



## APOPTOSIS

## FEATURED PARTICIPANTS

**Aposense**  
www.aposense.com

**BioVision**  
www.biovision.com

**Cambrex**  
www.cambrex.com

**Clontech**  
www.clontech.com

**EMD Millipore**  
www.millipore.com

**ImmunoChemistry Technologies**  
www.immunochemistry.com

**Life Technologies**  
www.lifetechnologies.com

**Promega**  
www.promega.com

**Sigma-Aldrich**  
www.sigmaaldrich.com

as biotin or fluorescein, because of its smaller size. To detect the molecule, an azide-coupled fluorophore is then fused to the alkyne through a copper-catalyzed click chemistry reaction. As a result, the Click-iT assay can detect breaks in DNA strands that other approaches—using larger-sized tags—might miss.

## COMPLEX SYSTEMS

Looking at what happens throughout an entire cell or organism can give researchers a more complete and accurate picture of apoptosis than a single assay, or even a time-lapse approach.

Already offering reagents for flow cytometry and developing multiplexing assays, Sigma-Aldrich is refocusing its efforts towards creating genetically altered cell and animal models that can be used to analyze the many components of the apoptosis pathway. These models are created using their CompoZr zinc finger nuclease technology, which act as highly specific scissors to create double-stranded DNA breaks for targeted gene deletion or insertion.

"It's less about specific reagent kits and more about developing the right model systems to study the genetic basis of apoptosis," says Helge Bastian, vice president of Global Marketing for Research Biotech at Sigma-Aldrich. Bastian says this approach came about because the company was not content to just keep improving the ease of sample preparation and making technical workflow improvements via speeding processing through ever-larger multiplexing approaches. Now, they are geared more towards providing products that are designed to specifically analyze genes and proteins, or the pathways in which they function.

The next frontier for probing apoptosis in complex systems is real-time, in vivo imaging. This effort is already underway for animal research, and companies are racing to deliver techniques that will also work in human clinical studies.

Promega has developed a bioluminescent caspase-3/7 probe for in vivo animal imaging of apoptosis. However, this requires the use of the luciferase gene, either by using genetically modified mice that express the gene or by surgically implanting tumor cells containing the luciferase gene, which is commonly used for screening potential cancer drug candidates.

**Aposense**, an Israeli-based company, has generated a new class of rationally designed, small molecular probes that detect cell membrane alterations that are indicative of apoptosis, including loss of membrane potential, exposure of phosphatidylserine (PS), and phospholipid scrambling. On such molecule, Apo-TRACE, can be used for pre-clinical in vivo imaging studies. Aposense has also developed an agent for PET (positron emission tomography) imaging in patients. The [18F]-ML-10 imaging agent, which is labeled with the radioisotope fluorine-18 (<sup>18</sup>F), is currently in clinical trials at multiple leading cancer centers in the United States. This cutting-edge technology may enable clinicians to see in real-time whether chemotherapy treatments that target apoptotic pathways work, and possibly aid in the individualization of patient treatment plans. Personalized cancer treatment is one the major unmet needs in oncology today, says Yoram Ashery, CEO of Aposense.

Providing in vivo imaging for the apoptosis pathway will be challenging because it will require a small molecule, nontoxic approach, unlike the in vitro techniques, says Gary Johnson, president of **ImmunoChemistry Technologies** (ICT) in Bloomington, Minneapolis. ICT is also trying to meet this challenge with tracers designed to detect the intracellular process of apoptosis, explains Johnson. One such molecule, labeled with the <sup>18</sup>F radioactive positron emitter, is undergoing testing for use with PET imaging in animals, and is anticipated to move into clinical trials by 2011. ICT is also developing tracers labeled with iodine-123 (<sup>123</sup>I) for single positron emission computational tomography (SPECT) imaging, which emits gamma rays, rather than positrons, to produce a 3-D picture.

The potential is great, says Johnson, because physicians will be able to determine if therapeutic treatments are working in less time than is currently possible, and without having to perform biopsies. Practitioners may also be able to use the technique to immediately assess the impact of neurological injury, and even degenerative diseases.

If Aposense's and ImmunoChemistry Technologies' approaches make it through clinical trials, researchers can expect another wave of apoptosis detection technologies, potentially including real-time "movies" that could help clinicians personalize treatments for patients, and watch whether and how these treatments work. This would move apoptosis detection off the bench and into the clinic—and greatly expand utility of tracking this complex, but key, process.

*Paul Smaglik is a freelance writer living in Milwaukee, Wisconsin.*

DOI: 10.1126/science.opms.p1000050



### MICROPLATE READER

The Synergy H1 Hybrid is offered as a highly flexible, high value monochromator-based multi-mode microplate reader for ultraviolet-visible absorbance, top and bottom fluorescence, and luminescence assays. The modular architecture can be easily upgraded to a “hybrid” reader with the addition of a high performance filter module, offering true versatility and unlimited assay use. This independent filter module with dichroic mirrors supports fluorescence polarization (FP), time-resolved fluorescence (TRF), TR-FRET, and BRET assays with ultrahigh sensitivity. Synergy H1 includes the Gen5 Data Analysis Software for advanced reader control, data analysis, graphing, exporting, and reporting. Additionally, Synergy H1 is compatible with the Take3 Multi-Volume Plate that measures up to sixteen 2- $\mu$ l samples, two BioCells, or a standard cuvette for fast and simple multivolume, multisample analysis.

BioTek Instruments, Inc.

For info: 888-451-5171 | [www.biotek.com](http://www.biotek.com)

### CELL HEALTH ANALYSIS

FlowCelect kits are designed for cell health analysis using flow cytometry and allow researchers to quickly and easily evaluate mitochondrial health in drug compound screening, apoptosis-related research, and understanding the mechanism of diseases. The six new kits include the MitoDamage kit, which provides simultaneous information on mitochondrial perturbations, apoptosis, and cell death. The MitoStress kit allows study of the interrelationship between mitochondrial oxidative stress and apoptosis. For researchers who want to further understand mechanistic pathways, Cytochrome C kits allow for evaluation of mitochondrial Cytochrome C levels in apoptotic cells and commitment to the intrinsic pathway of death, thereby simplifying an assay that has traditionally been done with Western blots. The kits may be used on any flow cytometer equipped with blue and red lasers.

EMD Millipore

For info: 800-645-54767 | [www.millipore.com/mitohealth](http://www.millipore.com/mitohealth)

### LOW EVAPORATION PLATES

Combining advanced optical properties with a low evaporation rate, Nunc Edge Plates enable researchers to obtain quality data from automated fluorescence imaging and quantitative analysis protocols. These plates incorporate large perimeter evaporative buffer zones that eliminate well-to-well variability, while dramatically reducing the overall plate evaporation rate to less than two percent after seven days of incubation. As a result, more viable and healthy cell yields are obtained. These zones enable the use of all 96 wells, greatly reducing the edge effect commonly experienced in cell culture, while maintaining data consistency throughout the plate. Nunc Edge Plates significantly reduce volume loss, effectively maintaining sample concentrations over long periods of incubation. The prevention of cell death and toxicity in the plates' outer wells allows results to remain more true to the population phenotype for more efficient, high throughput analysis. Nunc Edge plates offer outstanding flatness and superior image quality, ideal for high content analysis applications and cell-based assays where multiple targets are simultaneously imaged.

Thermo Fisher Scientific

For info: 800-625-4327 | [www.thermoscientific.com/edgeplate](http://www.thermoscientific.com/edgeplate)

### MICROPATTERNED SUBSTRATES

Patterns of extracellular matrix proteins can control important cellular functions including mitosis, apoptosis, cell growth, stem cell differentiation, tissue growth and structure, and multicellular dynamics and interactions. The new BioWrite line and grid protein micropatterns on glass coverslips are designed for cell-based applications. The fibronectin patterns have feature sizes ranging from 15  $\mu$ m to 100  $\mu$ m, which restrict the sites of cellular adhesion and spreading. The protein patterns can define the location, size, and morphology of cultured cells; control cellular functions; direct migration; and minimize variability in cell-based assays. The Substrate Design Center enables researchers to rapidly develop custom protein micropatterns with features as small as 5  $\mu$ m for specific cell-based applications. These products and services provide improved sensitivity, lower variability, and simpler automated image analysis for next generation cell-based assays.

Intelligent Substrates

For info: 410-982-6609 | [www.intelligentsubstrates.com](http://www.intelligentsubstrates.com)

### CELL CULTURE SEPARATION

FlexiPERM reusable silicone inserts can be used to subdivide slides and any cell culture dish into smaller cultivation units. Different cell lines can be grown in one vessel or equivalent cell lines can be grown in parallel with the assurance of comparable growth conditions. FlexiPERM inserts are highly adhesive to any flat surface for up to 14 days without additional—potentially contaminating—adhesives. After use, these inserts are easily removed without tools and may be sterilized and reused at least 10 times. Five versions of flexiPERM inserts are available. The flexiPERM slide and the flexiPERM micro 12 are ideally sized for microscope slides and feature eight and 12 cultivation wells, respectively. The flexiPERM disc provides four cultivation units and is appropriate for use with 60 mm dishes. FlexiPERM conA and conB inserts are single cone-shaped chambers suitable for micromanipulation and micro-injection applications.

Sarstedt

For info: 800-257-5101 | [www.sarstedt.com](http://www.sarstedt.com)

Electronically submit your new product description or product literature information! Go to [www.sciencemag.org/products/newproducts.dtl](http://www.sciencemag.org/products/newproducts.dtl) for more information.

Newly offered instrumentation, apparatus, and laboratory materials of interest to researchers in all disciplines in academic, industrial, and governmental organizations are featured in this space. Emphasis is given to purpose, chief characteristics, and availability of products and materials. Endorsement by *Science* or AAAS of any products or materials mentioned is not implied. Additional information may be obtained from the manufacturer or supplier.

# INTRODUCING AAAS **MemberCentral**



The exclusive new website for the AAAS member community.

AAAS MemberCentral is a new website focused on helping you — the scientists, engineers, educators, students, policymakers, and concerned citizens who make up the AAAS community — connect like never before.

On MemberCentral you can contribute to discussion groups or blogs, participate in a webinar, or share photos of your field research. You can exchange ideas, learn about your fellow members, and gain fresh insights into issues that matter to you the most. MemberCentral is also an easy access point for a wide variety of other AAAS membership benefits, like discounts on cars and books, travel opportunities, and more.

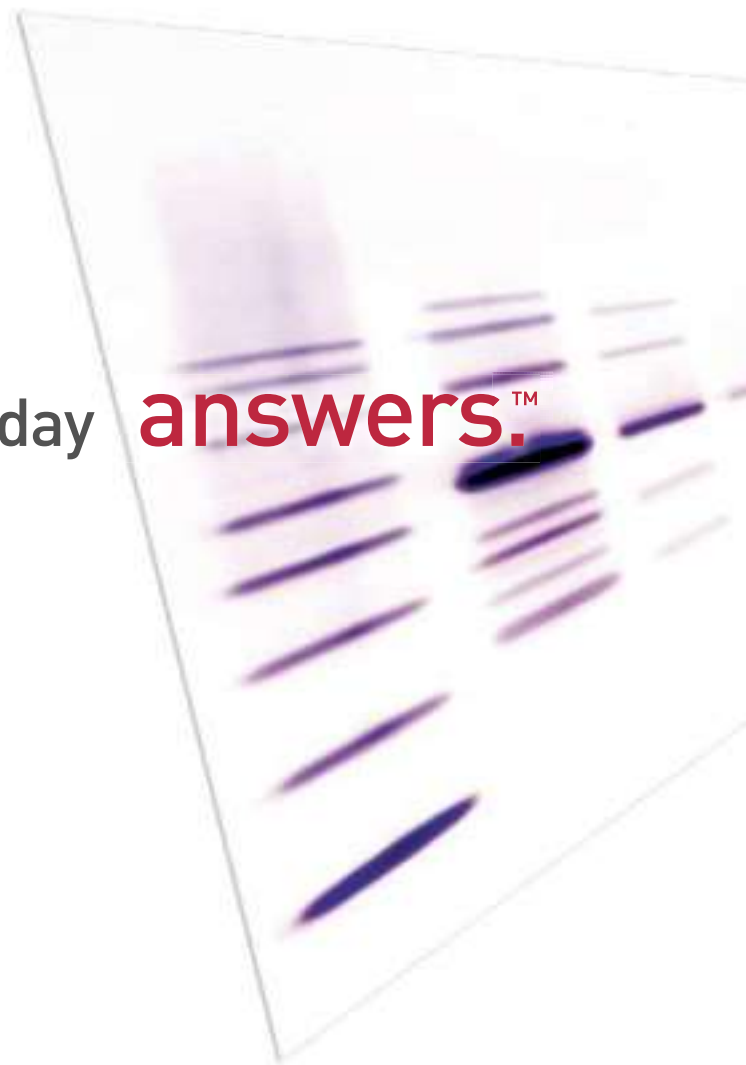
Experience MemberCentral for yourself. Visit [MemberCentral.aaas.org](http://MemberCentral.aaas.org) today and log in using your *Science* online username and password.



[MemberCentral.aaas.org](http://MemberCentral.aaas.org)



Everyday needs. Everyday **answers.**<sup>TM</sup>



Easily find your favorite brands of reagents, kits, and benchtop devices at [www.invitrogen.com/everyday](http://www.invitrogen.com/everyday).

**Ambion®**  
RNAi and RNA sample prep

**Dynal®**  
Magnetic beads for separation

**Gateway®**  
Cloning and protein expression

**GIBCO®**  
Cell culture

**Lipofectamine™**  
Transfection reagents

**Molecular Probes®**  
Fluorescent dyes and probes

**Novex®**  
Protein separation and blotting

**SuperScript®**  
cDNA synthesis reagents

**TaqMan®**  
Real-time PCR

**TOPO®**  
PCR cloning

For research use only. Not intended for any animal or human therapeutic or diagnostic use, unless otherwise stated.

© 2010 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners. TaqMan is a registered trademark of Roche Molecular Systems, Inc. These products may be covered by one or more Limited Use Label Licenses (see the Invitrogen catalog or our website, [www.invitrogen.com](http://www.invitrogen.com)). By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. C013357 0510